Growing Mushrooms the Easy Way

Home Mushroom Cultivation with Hydrogen Peroxide

Volume I

R. **Rush Wayne**, Ph.D.

Introduction

When I first took an interest in **growing mushrooms**, I checked out a well-known **book** on mushroom cultivation from the library and eagerly read through it. But my interest soon turned to general discouragement as I read about all the equipment and procedures the **book** insisted were necessary to grow mushrooms without getting the cultures contaminated. I would need a sterile laboratory space with a laminar-flow hood fitted with electrostatic and HEPA filters and an ultraviolet light. This space would need a sterile air-lock entry way with a foot wash, and I would need to have special clothing to enter it, so that I could wash down the floors with chlorine bleach every day. My fruiting mushrooms would have to be grown in a separate building altogether, so as to avoid getting spores into the sterile laboratory. These fruiting cultures would have to be grown in specially designed plastic bags with microporous filter patches attached, so that the mushroom mycelium could get oxygen without letting mold spores or bacteria get in. Of course, I would need an autoclave or at least a specially designed pressure cooker to sterilize the media that went into these bags.

After considering these requirements briefly, I put aside the thought of **growing mushrooms**. I

wanayswn’aty a.b Foruotm to w gheat ta Il lc tohualtd e gqautihpemr,e mnty, ahnodu sI ef iwguorueldd Ib pe rao bdaebaltyh wtraapsn f’otr c mutu osuhtr ofoomr t hceu ljtoubr es. Neither my wife nor I are careful housekeepers. We have unabashed dust and clutter, and green and white fuzzy things can be found in and outside the refrigerator. Although I was skilled at sterile technique from my years as a graduate student in biochemistry, I didn’t think that would save me from the legions of eager contaminants that would surely dog my every movement should I attempt to grow anything so delectable as mushrooms.

Still, the thought of **growing mushrooms** didn’t disappear entirely. Instead, a year or so later, it resurfaced again in the form of a new idea. I had read that culture media used for growing orchid seeds could be rendered free of contaminants if hydrogen peroxide was added to the medium.

While the peroxide killed bacteria, yeast, and fungal spores, it left the orchid seeds viable because they contained enough peroxide-decomposing enzymes to protect themselves. Then the orchid seeds could be germinated and tended easily by relative beginners without the need for strict sterile technique.

So here was a question: could added peroxide be used to keep mushroom-growing media, like orchidseed media, free of contaminants? If it could, then perhaps mushroom growing could be made accessible to beginners, just like orchid seed germination. So I resolved to try it with mushroom mycelium.

What followed was a fairly complicated and non-linear process of learning about growing various mushrooms, experimenting with adding hydrogen peroxide, trying different concentrations, learning about different culture media and how they interacted with the mushrooms and the peroxide, trying various degrees and techniques of pasteurization and sterilization, going back over earlier ground with better pH measurements, experimenting with supplements, tracking down sources of contamination, tightening my procedures, and on and on, until I developed some fairly reliable guidelines for what I was doing. It all took far longer than I ever would have guessed. But the upshot of it was that, yes, mushroom growing can be made accessible to beginners without the need for sterile facilities, air filtration, or even a pressure cooker, if one adds hydrogen peroxide to help keep the mushroom culture media free of contaminants. Using the techniques I developed, all the stages of mushroom culture can now be carried out by relative beginners, with a wide variety of mushrooms, and without investing a small fortune in equipment and facilities.

I have written the current **book** as a guide for the home hobbyist interested in mushroom growing, one that could serve as a basic stand-alone primer on home cultivation of several delectable mushroom species, and one that anyone can use, including the beginner. My previous manual, **Growing Mushrooms** with Hydrogen Peroxide, was written for the mushroom grower who is already familiar with traditional methods of mushroom cultivation, and its focus is on the use of hydrogen peroxide as an improvement to the traditional methods. While that manual made it possible for the first time to perform all phases of gourmet mushroom cultivation in the home without sterile facilities and air filtration, the current **book** goes even further and presents procedures that do not require pressure sterilization.

Of course, not all of the procedures described in this **book** were created by me. In particular, any procedures not specifically required for using the peroxide technique, or not specifically made possible by the peroxide technique, are likely to have originated elsewhere.

For in depth accounts of the traditional methods of mushroom cultivation, as well as the growth requirements for a wide range of mushroom species, please consult Stamets, Growing Gourmet and Medicinal Mushrooms, Chilton and Stamets, The Mushroom Cultivator, or another basic text. These books are valuable reference volumes for anyone who seriously wants to pursue mushroom growing in detail. Also, as you glance through these books, with page after page of discussion of elaborate sterile procedures and sources of contamination, you will enhance your appreciation for the simple techniques contained in this manual.

Note that sterile or aseptic technique, which the procedures in this **book** require to some extent, is always better demonstrated than described. It is my hope that the reader will seek out direct instruction in this regard. Your local mycological society may be helpful to that end.

Also note that this **book** is not intended as a guide for commercial cultivation of mushrooms, although the methods described here may well prove valuable to small scale commercial growers as well as to home hobbyists.

Preliminaries

The Mushrooms

Just about any mushroom that is currently cultivated can be grown in the home. However, some are easier to grow than others, and some, though **easy**, are not as rewarding as others that are more difficult.

I currently focus on four mushrooms in my own home growing. These are:

Hypsizygus ulmarius, the elm oyster or white elm mushroom: although it is not a member of the oyster mushroom family, it is an oyster-style mushroom in appearance and habit. It grows aggressively on sawdust or straw, it rarely has contamination problems following the techniques described here, and it does well in a variety of conditions and temperatures, fruiting either vertically or horizontally. When well cultivated, the mushrooms are large and attractive, rather like strange white flowers, and they are in my opinion the most delicious of the oyster-style mushrooms (not counting P. eryngii).

Pleurotus eryngii or King oyster: a member of the oyster mushroom family, but it does not have the usual oyster mushroom appearance or habit. A native of Europe, it grows up from the ground in a regal stance, rather than on trees and logs. It is large and thick-fleshed. Its substrate requirements are more narrow than other oysters, as are its temperature requirements. I have read that it prefers sawdust to straw, although I have not experimented enough with it on straw to confirm or deny this. It fruits best in fall or spring temperatures, growing at a glacial pace in

the cold of winter, and dying back in the hotter parts of the summer. It is one of the most delicious of cultivated mushrooms if cooked properly, sauteed rapidly in a wide pan, without being allowed to stew in its own juice, then lightly salted.

Hericium erinaceus or Lion's Mane, also called Pom Pom mushroom: a fungus that lacks the stalk and cap of a traditional grocery store mushroom, instead appearing as a kind of snowball covered with white icicles. It grows rapidly on sawdust substrates, and fruits easily over a range of temperatures. I have heard that it can be grown on straw as well, although I have never tried it. Chefs love this mushroom, and indeed it has a delicious gourmet flavor sometimes tasting like lobster.

Agaricus subrufescens, or almond mushroom: a member of the family that includes the domestic button mushroom and the Portabellas. It is distinguished by its unmistakable flavor of almond extract. Like the domestic mushroom, it prefers to grow on compost, but it can also be grown on straw, wood chips, or supplemented sawdust. It is a warm weather mushroom, but it will also fruit indoors in the winter in a heated room, making it a good candidate for year-round cultivation. This mushroom generally needs a casing layer--a layer of friable, soil-like mixture usually containing peat--applied to the surface of the culture in order to form fruiting bodies.

Some other mushrooms to consider include:

Lentinula edodes or Shiitake: ever popular, grown by many people and written about extensively elsewhere. I am not a shiitake grower, but the methods of culture handling, spawn and substrate preparation described here will all work for shiitake as well as for the mushrooms that I normally grow. Be sure to get a shiitake strain selected for growth on sawdust if you decide to grow this species using pellet fuel as your bulk substrate. Warm weather and cool weather strains are available.

Pleurotus ostreatus and other oyster mushroom species: like H. ulmarius, these are among the easiest mushrooms to grow, racing through sawdust or straw or any of a variety of other substrates. They were the first mushrooms I fruited using the peroxide method. Strains exist for most temperature ranges. The spores of P. ostreatus, which are released in great quantity from mature fruiting bodies, can cause health problems.

Ganoderma lucidum or Reishi: a top flight medicinal mushroom with immune stimulating properties. This mushroom grows on hardwood sawdust in warm temperatures. A related species from the Pacific Northwest, Ganoderma oregonense, prefers cooler temperatures. The woody mushrooms are broken up and made into tea.

Coprinus comatus or Shaggy Mane: a mild tasting, short-lived mushroom that grows best on compost.

I never got it to fruit indoors, but after I discarded the cultures in my yard, it appeared in my garden for a couple of seasons.

Hypsizygus tessulatus, or Shimeji: a cute, small round mushroom with a crunchy texture, grows on straw or sawdust-based substrates. The strain I bought required near freezing temperatures to initiate fruiting, so I didn't experiment much with it, but there are presumably others that will fruit without that.

Stropharia rugosa-annulata, or King Stropharia: a large mushroom that grows on beds of wood chips or straw and requires a casing and warm weather to fruit. The mycelium grows slowly, and only one variety is currently known to fruit indoors without regards to season of the year.

Agaricus bisporus/Agaricus brunnescens, or the white button mushroom, also the brown button mushroom, crimini and portobellos: so many button mushrooms are now grown by large commercial farms in the US, and sold so cheaply, that these companies can no longer make a profit. Like the almond mushroom, the preferred growth substrate for button mushrooms and portobellos is compost. Preparation of quality compost is a complicated and labor-intensive process that is beyond the scope of this manual. But button mushrooms can also be grown on straw prepared by the peroxide method (see Volume II). The yield will not be as high as on compost, but straw is so much easier to prepare at home that you probably won't miss the extra yield. As with the almond mushroom, a casing layer is required for fruiting, but button mushrooms require cooler conditions. Spawn can be prepared with pressure cooked grain (this volume) or with steamed instant rice (see Volume II, "Ten Minute Grain Spawn").

Equipment You Will Need

Some materials and equipment you may need for the peroxide method.

The methods described in this manual require very little in the way of equipment for growing your own mushrooms at home. Handling and measuring hydrogen peroxide requires only a measuring pipette (10 ml volume) and a graduated cylinder (probably 100 or 250 ml volume). These can be purchased from scientific supply stores. To measure the peroxide concentration in the bottles you get from the drug store, you will also need a small test tube with a lip, and a balloon. Preparing mushrooms spawn requires jars with lids (pint, 26 oz, or quart jars), a covered pot for steaming big enough to hold the jars, a small scale or balance for weighing, and some clear plastic food storage bags. Preparing agar cultures requires in addition a set of petri dishes. I recommend

reusable plastic petri dishes if you can find them. I purchased mine at my local scientific supply store. A pressure cooker, although not absolutely necessary, will be useful. These can often be found used at second hand stores that carry kitchen implements, or new in the kitchenware section of hardware stores and department stores. Make sure the cooker you get is tall enough to accommodate your jars. You will NOT need a glove box, HEPA filters, ultraviolet lights, a sterile laboratory, laminar flow hoods, air locks, foot washes, etc. etc.

For growing out the bulk substrate, you'll also need some small boxes (usually no bigger than about 500 cubic inches, or 8"x8"x8") and some fresh 0.5 mil or less high density tall kitchen bags (avoid the thicker soft plastic bags), or a set of 2 to 3 gallon plastic buckets with lids.
For helping the mushrooms along, you'll need a hand mister, and a cool space. Later, if you are growing a lot of mushrooms, you may want a fan and an automatic misting system.

Specialized Supplies You May Need

For making agar medium, you will need agar, light malt powder, and (if you plan to pressure cook your medium) yeast extract flakes, among other things. Agar is available at some health food stores, or through scientific supply houses, or commercial mushroom supply dealers. Note that although agar by itself is more expensive than ready-mixed MYA medium, the latter is only half or less agar by weight, so it is not necessarily a better deal. Malt powder is available from home brew supply stores or scientific supply houses. Yeast extract flakes are available from health food stores.
For spawn making and bulk substrate, you may need paper fiber pellets and wood pellet fuel. The paper fiber pellets are sold in my area as Crown™ Animal Bedding or Good Mews™ Cat Litter. (Check grocery stores, pet supply stores, farm and garden supply stores, etc.) In rural areas of the U.S. and Canada, wood fuel pellets can be found in grocery stores, hardware stores, farm supply stores, stores that sell pellet stoves, etc. In urban areas, check your phone **book** for distributors of wood pellet stoves, or check with your rural friends. It may take a drive out of town to get some. Try to find out what kind of wood they are made from, and look for hardwood for most mushrooms (fir pellets, however, will work well for P. eryngii and A. subrufescens).

The Basics on Peroxide

What peroxide does

The peroxide radical is a reactive form of oxygen which attacks various organic compounds. In living cells, it damages the genetic material, cell membranes, and whatever else it finds to react with. By doing so, peroxide in sufficient concentration can kill bacteria, bacterial endospores, yeast, and spores of fungi, including mushroom spores. It apparently can also kill small airborne particles of fungi, and the contaminants associated with human skin cells, which are said to be continually flaking off of the mushroom cultivator. Hydrogen peroxide thus acts to some extent against all commonly-encountered airborne contaminants of mushroom culture, including mushroom spores themselves. By contrast, antibiotics generally act only against bacterial contamination, and fungicides act only against yeasts and fungi.

The beauty of peroxide is that it does not kill established mushroom mycelium or interfere with its growth and fruiting. Despite peroxide's wide range of action against the common contaminants of mushroom culture, there is a relatively wide range of concentrations at which peroxide will allow the growth and fruiting of mushroom mycelium. The established mycelium, because of its ability to produce high levels of peroxide-decomposing enzymes, is evidently able to defend itself against much higher concentrations of peroxide radical than can isolated spores, cells or tiny fragments of multicellular organisms. So we can add hydrogen peroxide to mushroom cultures, and the mycelium will grow but the small contaminants will die.

This arrangement has a number of advantages. Most obvious is that it reduces the need for costly and elaborate facilities and equipment for environmental contaminant control. By adding hydrogen peroxide to mushroom culture media, it becomes possible to perform all phases of traditional mushroom cultivation, from isolation to fruiting, successfully in non-sterile environments with unfiltered air. Gone is the need for special clean rooms, HEPA filters, pre-filters, laminar-flow hoods, UV lights, air locks, glove boxes, or any other equipment associated with environmental control of microbial contamination--even microporous filters on bags and jar lids become superfluous. Using peroxide, the equipment minimally required for contamination control comes down to some measuring implements, a source of boiling water, and a large pot for steaming (or a pressure cooker for added security) --little more elaborate than is found in many kitchens. And whereas the traditional methods of mushroom culture required skillful sterile technique and immaculate personal cleanliness for success with agar cultures and spawn, use of peroxide allows success with only modest sterile technique and only minimal attention to personal hygiene. What' s more, it becomes possible to fruit mushrooms--even those that have the highest spore load--in the same building used to maintain agar cultures and grow spawn, without the fear that spores released by the fruiting bodies will diffuse into the agar cultures and ruin them. Hydrogen

peroxide uniquely will kill the spores of the very same mushrooms whose mycelium it protects. Do contaminants develop resistance to peroxide, the way they do to ordinary antibiotics? Yes and no. Many of the contaminants are already resistant to peroxide, and once they have established a colony, they will grow vigorously. Live Aspergillus (blue green) mold is very resistant to peroxide. But evidently peroxide at sufficient concentration overwhelms the resistance mechanisms of the single-celled organisms and isolated spores, and those of very small, isolated multicellular organisms as well.

What peroxide does not do

One thing peroxide does NOT do is eliminate all need for concern about sterile technique. Multicellular organisms and high concentrations of germinated spores are able to produce enough peroxide-decomposing enzymes to defend themselves against high concentrations of external peroxide. And since both multicellular organisms and concentrations of spores can be microscopic and reside on your hands or on particles of dirt or dust, you still have to take sensible precautions to keep your hands and all non-sterile particulate matter out of your early-stage cultures, even with peroxide added. Although you don't have to be afraid to leave cultures open to the air for brief times, to perform manipulations or otherwise check on them, you'll still want to use common sense in avoiding contamination. You wouldn't want to use the lid to a petri dish after you dropped it on the floor, for instance. Neither would you want to allow visible, non-sterile debris of any sort to fall into your cultures, or insects of any kind to enter them.
It is a good idea to periodically wipe the dust off shelves used to incubate cultures. You will still need to flame or otherwise sterilize whatever instrument you use to transfer chunks of mycelium from one culture to another. And I make it a regular practice to wipe my fingers with rubbing alcohol before performing inoculations of spawn or agar cultures. I do the same with any counter surfaces I use to perform manipulations with my petri dish cultures. This reduces the chances of larger particles making it into the cultures and helps protect the exposed mycelium.
It is also especially important to know and remember that peroxide does NOT protect the mushroom mycelium itself from aerobic contaminants. The mycelium decomposes peroxide that comes in contact with it, so any aerobic contaminants associated with the mycelium will be shielded from the deleterious effects of peroxide. Thus, as a general rule, peroxide only protects the culture medium or substrate from aerobic contamination. So your most careful procedure should be reserved for transferring mycelium, or performing any operation which exposes mycelium to unfiltered air.

And once your mycelium is contaminated, you will need to start over with a fresh, uncontaminated culture, or purify your mushroom tissue in some way to free it of contaminants. I'll discuss this more later.

Finally, peroxide is not a sterilant except at concentrations too high to use for mushroom growth. That is, you generally cannot use hydrogen peroxide by itself to sterilize culture media or mushroom substrates. At the concentrations that are compatible with mushroom growth, hydrogen peroxide will not kill live mold contaminants resident in the medium, and the peroxide itself will be rapidly destroyed by the peroxide-decomposing enzymes in non-sterile organic materials. Although some spores and bacteria may be killed by adding peroxide to non-sterile medium, there will be far more contaminants that will easily survive and grow within a short time. Therefore the general rule is: all culture materials and containers must be pasteurized before adding peroxide or peroxide-containing medium to them; culture materials that contain raw, unprocessed organic matter must be pressure-sterilized to destroy the peroxide-decomposing enzymes. And a corollary: any water used without pressure-sterilization in peroxide-treated medium should be clear and free of obvious particles, since any bits of organic or even inorganic material introduced with the water could harbor live contamination and/or peroxide-decomposing enzymes that would not be destroyed by pasteurization.

Safety and environmental considerations for hydrogen peroxide

There are no special safety precautions required for handling 3% hydrogen peroxide. Its toxicity is very low, and it decomposes completely to water and oxygen when it is spilled or ingested. It is odorless and does not stain or burn. It is generally not even active as a bleach until it reaches 60 oC, the temperature of very hot tap water. Every evidence suggests that it is environmentally benign. Since commercial peroxide is prepared chemically, rather than extracted from natural sources, it probably would not be considered compatible with organic certification standards following the criteria currently in vogue. However, I consider the use of peroxide to be in the spirit of organic cultivation. Since the peroxide added to mushroom cultures decomposes entirely to water and oxygen as the mushroom mycelium occupies the substrate, there can be no trace of the added peroxide left in the mushroom crop, beyond what is naturally there due to metabolic processes. Moreover, hydrogen peroxide itself is found naturally in all aerobic living organisms and in a variety of natural environments. From time immemorial, honeybees have secreted enzymes which add peroxide to their nectar, protecting it from bacteria, yeasts, and mold, and imparting

antibacterial properties to the resulting honey. The mycelia of at least certain mushrooms produce their own peroxide to help break down the woody substrates the organisms encounter. And peroxide is even a part of the healing defenses of the human organism. Indeed, around the world, thousands of proponents of a system of healing called oxygen therapy actually ingest food-grade peroxide solution on a daily basis to cure various ills and promote vitality, and some people have done so for many years (I do not necessarily recommend this, however). Finally, the use of peroxide circumvents the need for resource-intensive equipment, facilities and supplies, simplifying every stage of the mushroom cultivation process.

There is some question as to the effect peroxide oxidation may have on the mushroom substrate itself. Chlorine, when it reacts with organic materials like paper pulp, produces small amounts of dioxin, a very dangerous, cancer-causing chemical. Hydrogen peroxide does not produce dioxin, and as a result, environmentalists are campaigning to get paper companies to bleach their paper fiber with peroxide rather than chlorine. Still, it is conceivable that peroxide could produce some other harmful substance when it reacts with the organic materials in mushroom substrates. I have not ruled out this possibility, but I consider it unlikely. For one thing, aerobic living organisms have evolved for millions of years with hydrogen peroxide both in and around them. Peroxide is generated by normal aerobic metabolism, and it is also naturally formed by the reaction of water with oxygen in response to the ultraviolet rays in sunlight. This means that aerobic organisms most likely have developed metabolic machinery to deal safely with the variety of oxidation products that result from the reaction of peroxide with biological materials. In addition, hydrogen peroxide is chemically quite stable in sterilized mushroom substrates, and the concentration of peroxide we're using is so low that the amount of substrate oxidation going on has to be very low indeed. Finally, I have seen absolutely no evidence of any mutagenic or toxic effect of peroxide-treated mushroom substrate on the mycelium or fruiting bodies. Agar cultures containing hydrogen peroxide give fine, healthy halos of mycelium, and the final fruiting cultures produce mushrooms as beautiful as any grown by traditional methods.

Stability

The 3% hydrogen peroxide solution available at supermarkets and drug stores, with phosphoric acid stabilizer added, is quite stable on the shelf when kept relatively cool. When added to heatsterilized and cooled mushroom culture media, hydrogen peroxide evidently decomposes only slowly. Precisely how long it will last is presumably a complex function of media composition, peroxide

concentration, and temperature. However, my experience so far is that peroxide continues to exclude contaminants for long enough to allow the mycelium of a variety of mushroom species to safely colonize their substrates.

On the other hand, hydrogen peroxide should generally not be added to hot culture media, unless you are going to add extra to compensate for losses from decomposition. Since peroxide becomes active as a bleach above 60 o C, it will decompose readily when in contact with complex organic materials at this temperature and above. So wait until your medium has cooled -- if not to room temperature, then at least to a temperature that is comfortable to the touch--before adding peroxide. In contrast to its behavior in pure solution or sterilized media, peroxide breaks down rapidly in the presence of peroxide-decomposing enzymes, as happens when you put peroxide on a wound. The broken skin cells and blood vessels of a wound contain peroxide-decomposing enzymes in abundance, and they rapidly break down peroxide solution and release oxygen bubbles. Similar enzymes, known as catalases and peroxidases, are found in all kinds of living or once-living material, unless it has been heat treated or extensively processed. So, uncooked grain, flour, sawdust, wood, etc. all will destroy peroxide in short order. This means that you will need to keep all such materials out of your stock peroxide solution. It also means that if you want to incorporate such materials into a culture medium, you have to be sure everything in that medium gets thoroughly heat-treated or cooked clear through to destroy peroxide-decomposing enzymes before you add peroxide.

I take several measures to guard the purity of my stock peroxide solution. When I am about to withdraw peroxide, I first wipe down the lid and upper part of the bottle with rubbing alcohol, to keep out particles that might contain live contaminants. Then I either free-pour to a pasteurized measuring vessel or I use a clean, pasteurized pipette with the mouth-end plugged with cotton to draw up the solution. Pipettes do not need to be autoclaved, but they should be at least steeped in boiling water (filled somewhat beyond the top graduation, but below the cotton plug) for a few minutes, then cooled, before using them to withdraw peroxide. A one hundred milliliter graduated cylinder makes a convenient vessel for steeping a 10 ml pipette in boiling water. The heat will kill any live organisms in the pipette, while the peroxide itself will kill remaining heat-resistant spores. I also take care never to set the peroxide bottle cap down unless I am certain it will not contact contamination.

Variations in peroxide concentration from commercial sources

One annoying fact of life when using peroxide is that the solution you get from the drugstore or supermarket, labeled as containing 3% hydrogen peroxide solution, U.S.P., may or may not actually contain a 3% solution. The concentration can vary considerably, both above and below 3%. You can protect yourself somewhat from buying "worn out" peroxide by looking for the expiration date on the bottle, and getting one with the latest date, if there is a date at all. (The bottles of peroxide I get list only the month of expiration, not the year). However, even the expiration date gives no absolute assurance that the concentration is really 3%. It is important, therefore, to have a way to measure the peroxide concentration in the solution. This can be readily done by decomposing a sample of the peroxide and measuring the released oxygen, which I do with a simple balloon technique.

Get a clean test tube (preferably one with a lip or screw cap), a small birthday-party type balloon, and a slice, small enough to fit into your test tube, of the stalk of any mushroom you have handy (for best results, use a young, rapidly growing mushroom and take a piece of stalk, trimming off the natural skin to expose plenty of broken cells). If you don’t have any mushrooms, a piece of banana or other skinned vegetable should do just as well). You will also need your peroxide solution, a rubber band, a pasteurized measuring pipette, a 100 ml graduated cylinder, and a pot of water.

1) With the pasteurized measuring pipette, withdraw 5 ml of the peroxide solution from the bottle and transfer it into the test tube.

2) Place the slice of mushroom in the upper part of the tube (don't let it slip into the peroxide yet).

3) Make sure the balloon is empty of air and stretch the mouth of the balloon over the mouth of the tube (tilt the tube to keep the slice of mushroom from slipping into the solution until the balloon is in place.

4) Put a rubber band around the mouth of the balloon on the tube, to keep gas from escaping as the pressure builds (I have found it most effective to use a broken rubber band that can be wound tightly around the threads of the tube, over the mouth of the balloon).

5) Once the balloon is sealed in place, let the mushroom slice slip down into the peroxide solution. The solution should begin bubbling oxygen immediately.

6) Agitate the tube. The peroxide solution should be largely decomposed in five to ten minutes, depending on the amount of catalase/peroxidase in your mushroom slice.

7) When decomposition is almost complete, you'll see that the bubbling will have slowed and the

bubbles will have become quite small. Meanwhile, the balloon should have become taut as it began to fill with released oxygen.

Testing peroxide concentration

Now, my college chemistry training tells me that 5 mls of a 3% solution of hydrogen peroxide should generate about 49 mls of oxygen when the peroxide decomposes completely at room temperature and one atmosphere pressure. To measure the oxygen released from your peroxide solution:

1) Fill a graduated cylinder with water and turn it upside down in a pot of water, making sure all bubbles are out.
2) Twist the balloon on your test tube to trap the released oxygen, remove the balloon from the tube holding the twist tightly, and put the balloon under the water in your pot.
3) Carefully release the gas from the balloon up into the inverted graduated cylinder, displacing the water inside it.
4) Keeping the open end of the cylinder under water, read the volume of oxygen off the graduated cylinder.

Measuring released oxygen in an inverted graduated cylinder

The first time I did this, I got 52 mls of gas inside my graduated cylinder from 5 mls of peroxide solution. Given that there may well have been about 3 mls of air in the flat balloon before I started, the peroxide solution probably generated pretty close to the theoretical amount of oxygen for 5 mls of 3% solution.

Here's how to calculate the amount of peroxide solution you will need, if you solution tests higher or lower than 3%:

1) Divide the volume of oxygen expected for 5 mls of 3% solution (49 mls if the balloon is completely empty to begin with, or 52 mls in the above example, counting the few milliliters of air initially trapped in the balloon) by the volume of oxygen you actually got.
2) Multiply the previous number by the volume of peroxide solution you would add to your medium or substrate if it were really a 3% solution (this volume is given in appropriate section of this

manual, for instance, in the section on agar culture, you will find that you would need to add 6 mls of 3% peroxide for 1 liter of pressure-cooked agar medium).
Knowing the precise concentration of peroxide is most important when you are making agar plates (see below), since you will be working at concentrations close to the lower limit of effectiveness. When you are making spawn, you will be working at a considerably higher concentration, so there will be much more leeway for variation. I use less peroxide for bulk substrate than for spawn, but there is still some room for variation there, as well. I recommend you do the balloon test to check each new bottle of peroxide solution you use for making agar plates, and check the peroxide you use for making spawn and bulk substrate at least until you know how reliable your local product is. That way, you will know for sure that you are giving your cultures the protection you expect. Also, you may want to experiment with the peroxide sources in your local area to see who sells the most reliable product. Paradoxically, cheapest may be best, because there will be regular turnover of the stock where the price is lowest. If peroxide is not readily available at local stores where you live, you will probably want to order it from a chemical supply house. They will often carry 30% or 35% solution, which can be diluted. Swimming pool supply stores also may carry similar solutions. Note, however, that these concentrated solutions are considerably more hazardous than the standard 3% solution. Read all the precautions and warnings on the container and act accordingly.

Growing and Maintaining Agar Cultures

The first stage of mushroom growing is the propagation and maintenance of mushroom tissue (the mycelium) on agar as petri dish cultures. These first-stage cultures are used to store, propagate, and maintain the mushroom strains in a healthy state by serial transfer, and to inoculate the second stage cultures, the spawn.

Preparing agar plates

There are many recipes for agar medium that can be used to grow mushroom mycelium on petri dishes. I have tried several of these, but I currently use only one: malt yeast agar medium, also known as MYA. This medium has worked respectably for every mushroom species I have attempted to grow. It is not so rich that it contaminates instantly, yet most strains grow across a petri dish of MYA in two or three weeks. In my opinion, if you are using peroxide in your medium, there is

not much point to growing the mycelium any faster than that, since it will just force you to make up more agar plates sooner, to keep the mycelium fresh. Also, after repeatedly transferring the mycelium from plate to plate, some growers recommend that you start anew with mycelium from a storage culture, to avoid problems of senescence (aging) of the mycelium. The faster the mycelium grows, according to this view, the sooner one has to go back to storage. If this is true, I would just as soon have the mycelium grow relatively slowly.

I maintain all my petri dish cultures on peroxide-containing medium. Contamination on peroxide plates is rare, as long as a few precautions are followed, and you won't need to buy a laminar flow hood or build a glove box to keep contaminants out. You can pour your plates in the open air in your kitchen, and you can store and incubate your plates almost wherever you like, as long as the spot is relatively clean and the environment is compatible with mycelial growth. However, see my recommendations at the end of this section.

MYA Medium

Here is the recipe I use for one liter of MYA medium:

12 gms (0.42 oz) agar

12 gms (0.42 oz) light malt powder

1 gm ((0.035 oz) nutritional yeast powder

0.5 gm (0.017 oz) grain flour (I rotate between wheat, rye, corn, rice, oats, and millet)

0.5 gm (0.017 oz) rabbit feed (or other animal feed pellets)

4-5 gms wood fuel pellets (0.15 oz--the number of wood pellets can be increased for those wooddecomposing

species that do poorly on agar)

1 liter tap water

If you purchase ready-mixed MYA medium from a mushroom supply house, it will probably only contain the first three ingredients: agar, malt, and yeast. (You can add the others). Check the instructions to see how much of the powder the manufacturer recommends you use per liter of water. Usually it will be something like 40 to 50 gms. Depending on the proportion of agar to malt powder, you should be able to cut the recommended usage in half and get a medium that is actually better for the long term health of your mushroom cultures.

I prepare the agar medium for plates as follows:

1) I add all the ingredients to a jar with the desired amount of water. The jar should hold about

twice the volume you will actually use, to keep the agar from boiling over when it cooks.
2) I adjust the pH with a little baking soda (my water is acidic, but you could use vinegar if yours is alkaline. Also, see my "Note on Measuring pH of Substrate" below in the section on preparing bulk substrate).
3) I then use my ordinary kitchen pressure cooker to melt and sterilize the medium. (I use tap water and have not had any problems with it. In fact, when I grew mushroom mycelium on medium prepared with distilled water, growth was noticeably slower). I put lids loosely in place and pressure cook at 15 psi for no more than 10 minutes, allowing an initial ten minutes of steaming to melt the agar before putting on the pressure regulator. (If you are using ready-mixed MYA medium, the instructions may say to pressure cook for much longer times, for example, 45 minutes. Don't do it! 20 minutes is the most youÕll need, and any longer will produce carmelization products in the medium that are harmful to the mycelium). I also sterilize a set of petri dishes along with my medium, placing the dishes in a large tomato can covered with aluminum foil (I use plastic reusable petri dishes, and a liter of medium fills up about 30 plates).
There is no need to avoid entry of unsterilized air, assuming there is not a great deal of heavy dust, since the peroxide will kill the airborne contaminants when it is added.
5) When I can handle the jar quite comfortably, I usually put the jar of agar in a pot of warm water for the last part of the cooling process, since the agar is close to solidifying at this temperature.
6) Then I add my peroxide solution with a pasteurized pipette and quickly mix the peroxide into the medium by moving the jar with a circular motion, reversing directions a couple of times (but doing my best to avoid making a lot of bubbles, which will end up on the surface of the agar).
7) Once I've added the peroxide, I go straight to my petri dishes, which I have set out on a clean counter, and I free-pour the medium into the dishes, closing the lids as I finish.
Pouring melted agar into Petri dishes
8) When the agar has solidified, I set aside the plates to dry for a few days in a lightly covered tray.
To be on the safe side with my plate cultures, I use the lowest concentration of peroxide that I have found effective in agar medium, which is about 0.018%, or 6 mls per liter of medium. (You can add twice this much without visible harm to the mycelium of the species I have grown, but note that very slow-growing species such as Stropharia may be more sensitive to peroxide exposure. The production of protective peroxide-decomposing enzymes seems to be roughly parallel to the rate of growth of the organism). When the plate is inoculated, the concentration

presumably begins to drop slowly below the initial level as the peroxide is decomposed by the spreading mycelium. Eventually, the peroxide should disappear completely when the agar is overgrown, if not earlier. Once this stage is reached, colonies of mold may begin to appear at the edge of the agar plate.

No pressure agar medium

If you do not own a pressure cooker, or do not want to use one, you can still make serviceable agar plates by boiling/steaming the agar medium, provided you alter the above recipe somewhat. You will need to replace the ingredients that contain peroxide-decomposing enzymes with other ingredients that are free of those enzymes. In the above recipe, agar, malt powder, and pellet fuel do not contain peroxide-decomposing enzymes, but yeast powder, flour, and rabbit chow all do. In order to use peroxide in our agar medium, we ordinarily have to pressure cook the medium to destroy the peroxide-decomposing enzymes in these ingredients. However, other ingredients can be used in their place. The yeast powder provides vitamins, so this ingredient can be replaced by a bit of a fresh synthetic B-complex vitamin pill. Because it is synthetic, it will not contain enzymes. Grain flour and rabbit chow provide protein/nitrogen, so these ingredients should be replaced by other peroxide-compatible protein sources. Typically, only highly processed substances are free of peroxide decomposing enzymes, substances like gelatin, soy milk powder, non-fat milk powder, low sodium soy sauce, etc. To test for the presence of the enzymes, mix a little of the substance in question with some 3% peroxide solution and watch for evolution of bubbles. No bubbles means you are in the clear.

Here then is a recipe for one liter of "No pressure" agar medium:

12 gms agar
12 gms light malt powder
0.5 gm processed nitrogen source (rotate between gelatin, soy milk powder, milk powder, low sodium soy sauce, etc.)
5-7 wood fuel pellets
small chip (0.1 gm? enough to turn the solution slightly yellow) from synthetic B vitamin complex pill
1 liter clear water, free of particulates

1) Let your jar containing this medium (and your petri dishes, if reusable) sit in boiling water with lid on for atleast 45 minutes.

2) Then remove the jar and let it cool, adding the peroxide as in the first recipe. The peroxide will kill any spores remaining in the medium. I add slightly more peroxide to non-autoclaved plates, about 8 mls per liter of medium. In general I find that non-autoclaved peroxide plates contaminate more often than autoclaved peroxide plates, but they still do considerably better than plates made without peroxide.

Watch out for drips of agar medium that land on the outside of your petri dishes. If these are not cleaned off, they will grow mold within a few days, and the spores will diffuse into the plates and start germinating at the outer edge of the agar.

If you are working with reusable petri dishes as I am, clean them carefully after you take out the old agar. Even the smallest amount of old medium left in a plate, if it is not in contact with the peroxide in the new agar the next time you use the plate, can grow mold and become a jumping-off point for contamination.

A benefit of pouring plates when the agar is so cool, is that there is considerably less condensation on the inside of the lids, than if you pour hot. This obviously means that you won't have to take special measures to get rid of condensation, such as shaking out the lids, or warming the plates to evaporate the droplets. And you'll be better able to see what is happening in your plates. However, the agar surface still needs some drying, so I let the plates sit at room temperature for a day, lightly covered with a sheet of wax paper to keep dust off, before I use them. Plates with agar medium that has been steamed will be wetter than plates with medium that has been pressure cooked, because of the lower cooking temperatures and shorter cooking time, so they will need to be dried for a longer time.

If you have extra agar medium after your plates are poured, the excess will remain sterile stored in the refrigerator. When you get around to using it, you can melt the agar again, but note that you will need to add peroxide again, because the heat of melting will have destroyed what you added the first time.

Acquiring mushroom cultures

There are several ways to acquire a culture of mushroom mycelium to grow out on your agar plates. Spores from a mushroom can be germinated in nutrient medium. Tissue can be cut aseptically out of a fresh mushroom and coaxed to sprout mycelium on nutrient agar. Or a mushroom tissue culture can be purchased from a commercial supplier, usually in the form of an agar tube culture or a petri dish culture.

Since peroxide-containing nutrient medium kills mushroom spores, I have not worked with germinating spores to acquire mushroom cultures. Instead, I prefer to purchase tissue cultures from a reputable supplier. The way I see it, the supplier has already gone to the trouble of isolating a mushroom strain with desirable characteristics, and by purchasing a tissue culture, I am relatively assured of obtaining a mycelial isolate carrying the same desirable characteristics. By contrast, if you try to grow a mushroom strain you've isolated from spores or cloning and it doesn't fruit, you won't know whether it is the conditions you are providing, or the strain that is causing the problem. (Spores are like seeds: they may or may not have the same genetic characteristics as the parent). And you can waste enormous amounts of time trying to fruit a worthless strain. Moreover, once you work out the conditions for growing an isolate in your situation, if you ever lose the culture, unless you purchased from a commercial supplier, you can't go back to the same supplier and get another "copy." You'll have to start all over and work out the conditions again for a new strain.

When you purchase a tissue culture from a commercial supplier, it is generally understood that you will use that culture to grow--and sell if you choose to--spawn, fruiting cultures, and fruiting bodies of that mushroom strain. It is also presumed that you will not take that culture and use it to establish your own commercial strain bank, selling agar cultures to others. If you want to sell agar cultures, the ethical route is to isolate your own strains by cloning from the wild or germinating spores.

Cloning mushrooms

It can be fun, regardless, to clone your own mushroom culture from a specimen you collect in the wild. Perhaps it will provide you with a first-rate fruiting strain. If you'd like to try it, you will need some nutrient agar plates containing peroxide (see below), a scalpel, an ethanolfueled alcohol lamp, and a fresh mushroom.

To clone a mushroom:

1) Clean off the external surface so that there is no loose debris.
2) Break open the mushroom cap (or base of the stalk) as cleanly as you can.
3) Light your alcohol lamp and flame your scalpel blade. Then cut out a small piece of clean tissue within the mushroom that does not contact any external surface. This will obviously be easier with thick, fleshy mushrooms than with thin ones.

4) When you have piece of mushroom on your scalpel, transfer it to the middle of one of your nutrient agar plates. Since the chances of failure are high, take a few more pieces if you can and transfer each one to its own agar plate.
5) Finally, stack the plates, wrap them in a clear plastic bag, and set them in a convenient place to incubate at room temperature.
6) Mycelial growth, if it occurs, should become visible in a number of days, spreading out from the chunk of mushroom tissue. Mold or bacteria may grow as well, in which case you may have to cut out a bit of clean mycelium and transfer it to a fresh plate. If you decide to subclone the mycelium away from mold contaminants in this way, be sure you do it before the mold has matured enough to darken in color and form spores. Otherwise you will simply be transferring mold with the mycelium.
If you are trying to clone mushroom mycelium from the wild, remember that hydrogen peroxide in your medium will not by itself clean your mycelium of resident contaminants. If your material is dirty, and you can't get a piece of clean tissue by breaking open the stalk or cap of the mushroom you want to clone, peroxide in the agar will not improve matters. It is not a sterilant. However, if your material is basically clean, peroxide in your agar will at least reduce the incidence of background contamination on your cloning plates.

Strain storage

Once you have acquired a mushroom culture, you will need a way to safely preserve samples of it for long periods, so that you can go back to these preserved samples if anything happens to the active culture you use every day. The storage method I use simply requires scraping a bit of mycelium off an agar plate and transferring it to a screw cap tube of sterile distilled water (thanks to Joe Kish for bringing this technique to my attention). Once in the distilled water, the mushroom mycelium goes dormant and will last indefinitely for some strains (oyster-type mushrooms last only about a year in my experience). Refrigeration is not even required. Although agar slants are the "traditional" way to store cultures for those who don't have liquid nitrogen, slants do not preserve strains very long--six months at best.
Now, when you make storage preparations of strains you want to preserve for long term storage, I recommend that you prepare these without resort to hydrogen peroxide. The reason for this is that I don't really know what long term effects peroxide exposure may have on mushroom storage cultures. Could it accelerate senescence? Does it weaken the strains gradually? Are there gradual

genetic changes? I am simply not in a position to rule out all the problems that could occur with all the different species you may want to store. In addition, actively growing cultures are better able to defend themselves against added peroxide than dormant storage cultures, which may be more subject to damage. So, although slants and distilled water storage tubes can easily be prepared with peroxide, peroxide-free storage of strains is the safest course. Besides, it is so **easy** to prepare good clean distilled water storage tubes by dispensing the distilled water into the tubes, lightly screwing on the caps, then pressure cooking for half an hour (if you don't have a pressure cooker, I would try boiling for an hour with a few drops of 3% peroxide; the peroxide will kill the heat-resistant spores, and then the lengthy boiling will destroy the peroxide). And unlike petri dishes, screw cap tubes can be flamed on opening and closing, making it easier to keep them sterile without air filtration while inserting mycelium. I wrap the final storage tubes, containing mycelium, in clear plastic "food storage" bags before putting them in a safe spot in my basement.

Inoculating and handling agar cultures

I inoculate agar plates and slants by sterilizing a scalpel with the flame of my alcohol lamp, then transferring to each fresh plate or slant a small chunk of mycelium-impregnated agar cut from a plate that has a healthy halo of mycelium growing across it.

Transfering mycelium by way of an agar chunk.

When using a flamed scalpel to cut out agar chunks, I first cool the scalpel by plunging it into the agar of the plate containing the culture I want to transfer. (Traditionally, you would cool the scalpel by plunging it into the agar of the new, unused plate. But the hot scalpel may decompose some peroxide in the plate at the site of the cut. In unfiltered air, this spot then might become a locus for contaminant growth, since it is less protected. This is not a problem with the plate I inoculate from, since it will be discarded. But it may be a concern with the new plate. So I cool the scalpel in the old plate).
If you are inoculating a plate from a storage culture devoid of peroxide, don’t use an inoculating loop except to fish out a large clump of mycelium. In my experience, the minor mycelial fragments picked up by an inoculating loop are not enough to establish colonies readily in the presence of the concentrations of peroxide that are effective against contaminants,

especially when the culture has not been growing on peroxide previously. The mycelium has a much better chance of taking hold if you can transfer a clump of mycelium from a distilled water storage tube, or a chunk of mycelium-containing agar excised from a slant using a scalpel or other sharp sterile implement. (Admittedly, it is somewhat awkward and sometimes frustrating to dig pieces of agar out of a slant with a scalpel).

Cultures that have not been exposed to peroxide medium previously will often lag at first, as the mycelium adjusts to this new feature of its environment. Sometimes the mycelium will appear to be trying to grow away from the peroxide agar at first. (You may observe similar behavior when transferring from a plate that originally contained peroxide but that has been overgrown with mycelium for a few days so that all added peroxide has decomposed). Sooner or later, however, the mycelium will settle down and grow normally over the surface of the new medium.

I have never observed any problem with my strains which I could attribute to continuous peroxide exposure. Typically, I transfer my strains about ten times on peroxide-containing medium before returning to peroxide-free storage cultures, although the choice of ten transfers is an arbitrary one, and returning to storage cultures may not be necessary at all.

Note that peroxide protects only the portion of an agar plate that does not have mycelium growing on it. The mycelium itself is unprotected, since it decomposes the peroxide as it grows. Therefore, older plate cultures that have been overgrown with mycelium for more than a few days have an increased likelihood of harboring contaminants.

Preventing occult contamination with bottom inoculation

It is also possible for contaminants to accumulate on mycelium if you transfer it repeatedly in unfiltered air, even if there is always peroxide in the agar and the mycelium never covers the entire plate. Although you may never see contaminants growing on the mushroom mycelium in your plates, invisible contaminants will slowly build up. This "occult contamination" can be a problem whether or not you are using peroxide in your spawn and in your fruiting substrate as well. However, if your spawn or fruiting substrate will be peroxide free, there is an even greater chance that the occult contaminants could bloom when they enter the unprotected medium.

To guard against the possibility of such occult contamination, I use a simple trick: I regularly inoculate the bottom of the agar when I do my transfers (How often you do this depends on how you store your plates, and for how long. The safest course is to perform this operation at every transfer, at least with those plates used for transferring mycelium to subsequent plates. But you

may be able to get away with putting it off for two or three transfers before it starts affecting your success rate). I perform the bottom inoculation as follows:

1) Turn the plate upside down.

2) Lift one side of the plate bottom as if it were hinged to the top, and gently pry the agar disk out into the lid of the plate with a flamed scalpel. (If your agar regularly tears or breaks at this step, you will need to increase the amount of agar you add for making your medium.)

3) Close the plate back up until you're ready, then transfer a chunk of mycelium to the exposed underside of the agar with a flamed, cooled scalpel.

4) Finally, after inoculating the underside, close the plate, turn it right side up again, and gently pry (again with a flamed scalpel) the agar disk back to its usual position in the bottom of the plate, now sitting on top of the chunk of mycelium.

This arrangement forces the mycelium to grow up from the bottom of the agar through the medium to the surface of the plate, leaving any accumulated contaminants behind in the process. Certain strains may not respond well to this arrangement, but so far I have not had any problem as long as the strain I was using was able to grow vigorously on the medium. However, because of the amount of manipulation involved, this procedure does carry an increased risk of contamination compared to simple transfers. Whereas I rarely see contamination on peroxide plates inoculated in the usual way until they are old, I lose perhaps one in five plates inoculated on the underside of the agar. Wiping down your counter surfaces and your fingers with rubbing alcohol before you begin may help cut down on such failures.

A tricky but important point in the bottom-inoculation procedure is to avoid scraping bits of agar onto the rim of the petri dish bottom when you close the plate after inoculating the bottom of the agar. Bits of agar that get on the rim, or outside of it, tend to sprout contaminants because of their proximity to ambient air. It is also advisable to use plates that have been dried sufficiently to eliminate obvious surface drips. If the agar is still very wet when pried into the lid, it may leave enough medium behind in the lid to cause troubles at the edges of the plate later on.

One final point: Be sure not to cut all the way through the agar when you remove wedges for inoculation from a bottom-inoculated plate. Doing so will defeat the whole purpose of the procedure by bringing along the occult contaminants we’re trying to confine to the bottom of the plate. To leave these contaminants behind, excise wedges only from the top of the agar.

Once my agar plates are inoculated (I keep four at a time for each strain), I place them inside fresh clear-plastic food-storage bags, which I close with twist ties. The closed bag provides a

still-air environment and helps keep out marauding fungus gnats and mites. I put three or four petri dishes in a single bag. They then can be incubated anywhere that is convenient--on a bookshelf, in a closet, on a counter top, etc. However, I do not recommend storing plates in the refrigerator, because of the condensation that is produced, and I also don't recommend incubating plates on a shelf above a heater, because the on-off cycles of heating and cooling will cause contaminants to be drawn into your plates.

Being able to store fresh (uninoculated) plates easily is one of the benefits of peroxide. I keep a set of fresh plates stored in a cool spot--again, not the refrigerator. Like the inoculated plates, I keep these wrapped in plastic food storage bags. Each time I use one of my growing cultures to inoculate spawn, I also take out one of these fresh plates and inoculate it to replace the culture I've used. At the same time, I replace any cultures that may have developed mold colonies at the edge. This way, the number of plates I have growing remains constant, and I rarely find myself short.

Making Mushroom Spawn

Production of spawn is the second stage in mushroom growing. Spawn is the "starter" used to inoculate bulk fruiting substrates, or to make more spawn. Traditionally, spawn making was best left to commercial spawn suppliers, who had the sterile facilities to keep spawn free of contaminants. With the development of the peroxide method, however, spawn making is just another step in the mushroom growing process, and an **easy** one at that.

Being able to make your own spawn without a sterile facility has a significant economic benefit for the small grower or hobbyist. At $20 to $25 for a few pounds of spawn, purchasing spawn represents a significant expense. If you make the same few pounds yourself with the help of peroxide, grain will cost you about a dollar or two, and the peroxide -- ten cents. (Sawdust, if not free, will cost quite a bit less than grain). And you won't have to spend a small fortune building an air filtration set-up or special clean rooms for incubating the spawn.

Jars of peroxide-treated sawdust spawn on a bookshelf

For my own mushroom growing, I have switched almost entirely to using sawdust-based spawn. With my current methods, sawdust-based spawn medium can be prepared more quickly and easily than grain spawn, without soaking of grain, and even without autoclaving or pressure sterilizing (see below). Also, mature sawdust spawn colonizes sawdust-based substrate more quickly, and in my experience, with a lower incidence of mold contamination, than grain spawn. And contamination of

the spawn itself is rare, perhaps one jar in one hundred, as might be expected just from occasional, inevitable mistakes in technique. True, sawdust spawn does not contribute as much nutrition to the bulk substrate as grain, but it is not particularly difficult to add the missing nutrition directly to the substrate from other sources that do not require pressure cooking.
For most mushroom species, grain spawn is recommended for straw, since the grain adds to the nutritional base of the substrate. And that grain has to be pressure sterilized. Still, there are two good species that will grow well on straw using sawdust-based spawn instead of grain: Hypsizygus ulmarius (the elm oyster) and Hypsizygus tessulatus (Shimeji). Since H. ulmarius grows every bit as easily and tastes considerably better than traditional oyster mushrooms of the Pleurotus family, I can see no reason to incur the added difficulty of grain-spawn making simply to grow oyster mushrooms on straw.

Ten Minute Spawn

My own procedure for preparing sawdust-based spawn originally required sterilizing separately enough water to add diluted peroxide to the spawn after it had been pressure-cooked and cooled. This is an awkward procedure at best, so I sought alternatives. My search lead to the development of "10 minute spawn," a form of sawdust-paper pellet spawn that only requires a ten minute steaming and no pressure cooking at all. This is probably the fastest method yet in existence for preparing sawdust-type spawn. In this "one step" procedure, all the solid ingredients are placed in a jar, then peroxide is added with all of the water, and the spawn medium is briefly steamed and cooled. Enough peroxide evidently survives the brief steaming to keep the spawn contamination-free.

Here's the recipe for making "ten minute spawn:"

Paper Fiber pellets – 3 oz
Wood Fuel pellets – 1.5 oz
Ground limestone - 0.015 oz (0.4 gm, or about 1/4 teaspoon)
Gypsum (optional) - 0.015 oz (0.4 gm, or about 1/4 teaspoon)
Nitrogen supplement - 2% by weight (see below) - (usually about 3/4 tablespoon total)
Hot water - 150 mls, mixed with 3% hydrogen peroxide - 20 mls
Jar with lid containing fitted cardboard disk (see below under "Spawn Containers")

1) Place into a 26 oz or similar size jar the wood fuel pellets (ordinary sawdust will NOT work), paper pellets (Crown™ Animal Bedding or Good Mews™ Cat litter, etc.), lime, gypsum (optional)

and nitrogen supplement (see below). The wood fuel pellets must be made of a relatively light wood, such as cottonwood or fir, so that they disintegrate easily as well as heat up and cool down quickly. Fresh ground oyster shell lime will substitute for limestone.

2) Add the hot water with peroxide to the jar, and mix slightly.

3) After allowing a few minutes for the liquid to be absorbed and the wood fuel pellets to disintegrate, shake the jar with a temporary lid in place to mix the ingredients, then knock the jar on a padded surface to dislodge substrate from the upper part of the jar back down into the rest of the substrate.

4) Slightly moisten the cardboard disk in the final lid and put the lid loosely in place on the jar.

5) Place the jar in a steamer pot containing about a half inch to an inch of water, cover the pot with a fitted lid, bring the pot to steaming temperature, and let it cook for just ten minutes over a rolling boil. (I want the pot to reach steaming temperature quickly, so I start with hot tap water. The jars sit on a rack that elevates them slightly from the bottom).

6) When the ten minutes are up, withdraw the jar and let it cool rapidly to room temperature.

7) Wet the cardboard disk inside the jar lid with some 3% peroxide by free-pouring a little peroxide into the lid and wiggling the lid to spread the liquid. Pour off any excess.

8) The spawn is then ready to inoculate.

In the above procedure, I mix one part sawdust with about two parts pelletized paper. The pellets allow the spawn to break up on shaking in jars after the mycelium has grown through the substrate. Of course, you can also prepare your sawdust spawn in heat-resistant plastic bags, and then you won't need paper pellets, since you can break up the spawn by manipulating the bag.

Agar colonizes sawdust by itself with difficulty, which is why I have added an additional nitrogen source to my sawdust spawn in the procedure. The standard recipe calls for one part bran for every four parts sawdust, but if you use any such "raw" supplement as bran, you will have to pressure sterilize your spawn after all, to eliminate peroxide-decomposing enzymes. Therefore, I have identified several nitrogen supplements that do not require pressure sterilization.

Two readily available choices are powdered soy milk and powdered cows milk. I have used each of these substances successfully in the above recipe for 10 minute spawn by adding 0.3 oz (or a little less than a tablespoon) to the specified amounts of paper fiber pellets and wood pellet fuel.

Sylvan Corporation sells two processed supplements, one based on denatured soy protein (Millichamp 3000), and the other based on corn gluten (CG60), and these serve the purpose quite

well (I add 0.30 oz in the above recipe for "10 minute spawn"). Neither of these commercial supplements decomposes peroxide when the supplement is fresh, although older Millichamp 3000 and a third supplement sold by Sylvan, CS36, does.

Artificial fertilizer can also provide a workable nitrogen source (for example, about 0.1 oz of "Schultz Instant" brand 20-30-20 fertilizer works well in the above recipe). I have used this successfully with both P. eryngii and H. ulmarius. However, be forewarned that mushroom mycelium takes some time to adapt to chemicals such as these, so the growth will start off quite slowly.

Perhaps you don't like the idea of using artificial fertilizer. Well, since human urine contains nitrogen primarily in the form of urea, it can be used as an organic supplement in place of the fertilizer. In that case, you could replace roughly half of the water required with fresh urine.

To use other supplements, the idea is to add enough to bring the percent nitrogen in the spawn medium to about 0.4, or the percent protein to about 2.5. See the section on supplements under bulk substrate preparation for details of making this kind of calculation.

Two final notes on this ten-minute spawn procedure: first, be careful to use clean containers and implements, use only clear water, free of particulate matter, and if you are working in a kitchen, make sure you don't get flour, crumbs, or other organic matter into the jars or the containers you use for weighing out the medium. Also, make sure none of your ingredients (or your cardboard disks for the lids) is so old it has had a chance to get moist and start to decompose. This will introduce live contaminants containing active peroxide-decomposing enzymes. The procedure works because there are no peroxide-decomposing enzymes in any of the ingredients, so you need to ensure that this remains the case.

Second, the procedure also works because the small amount of material I am using for a 26 oz. jar can be heated and cooled quickly, so that some intact peroxide remains after the steam treatment. Larger quantities of spawn will both take longer to heat through and longer to cool, so they will likely require the addition of greater amounts of peroxide to assure that any survives. You will have to perform your own experiments to determine the amount of peroxide to add.

Pressure-sterilized sawdust spawn

If you are not going to use wood pellet fuel as a source of sawdust, or if you want to use an unprocessed nitrogen supplement like bran, you will have to pressure sterilize your sawdust spawn, and add diluted peroxide to the medium after it has cooled. You'll want to sterilize enough water separately to dilute the peroxide in about one-third to one-half the total water

added to the substrate. After measuring out the diluted peroxide you need, pour it into the spawn medium and then shake well to distribute the liquid.

Here's the procedure as I used to do it:

1) Add roughly half the total wateryou'll need to the spawn medium in as many containers as you want to prepare.
2) Measure out and sterilize enough water to add the other half of the total water, with peroxide, to each of the containers later.
3) Dilute your peroxide into the sterile water when it is cool, to make a 1 to 10 dilution (that is, add a volume of 3% peroxide that is roughly a tenth of the total volume of the water). 4) Measure out the individual amounts of water for each spawn container in a graduated cylinder pasteurized with boiling water.
5) Free-pour the measured water into each spawn container (resulting in an additional 1 to 2 dilution, since the containers already contained half of the water) making sure to wipe off drips running down the outside of the cylinder so they won't fall into the spawn during pouring, and mix immediately. The total dilution comes to about 1 to 20, or a peroxide concentration of roughly 0.15%, same as for grain spawn.

Grain Spawn

Now, if you have decided that you need grain spawn, I have to caution you--especially if you have never made grain spawn before--that making grain spawn can prove difficult even with peroxide addition. This is because the grain available to you locally may carry a high load of endogenous contaminants that cannot effectively be eliminated by pressure cooking.
So, although I have employed lengthy sterilization periods and plenty of peroxide, I have not been able to consistently make contamination-free rye spawn with the rye grain I get locally. Fortunately, I have been able to substitute a grain called soft white wheat. It has a much higher initial moisture content than rye berries (30% vs 8%), but for whatever reason it is much cleaner than the rye I can get. Soft white wheat has worked well for me when I have added a measured amount of hot water and let the grain stand overnight before pressure cooking, or when I have steeped the grain with excess hot water. I get contamination-free grain spawn virtually every time with this grain. Unfortunately, soft white wheat is sometimes unavailable, and store personnel are prone to mix it up with hard red wheat, a low moisture grain which gives me the

same problems as rye. Whatever grain you choose,you'll want to be sure that 1) your substrate is completely sterilized before you add peroxide, and 2) you have removed all traces of medium on the outside of your containers. Of course, the problem of thorough sterilization also exists in preparing spawn in filtered-air environments. If there are mold spores or bacteria inside the grain kernels or other substrate particles, and these are not killed by autoclaving/pressure cooking, they can germinate and spoil the spawn despite filtered air or added peroxide. With peroxide as well, however, incomplete sterilization means that some peroxide-decomposing enzymes are left in the grain, creating pockets of medium that are unprotected by peroxide.

The second problem also exists in conventional cultivation practice. If traces of culture medium get on the outside of culture containers, these bits of medium can become loci for contaminant growth and spore dispersal. If this happens with peroxide-protected substrate, the culture will often remain clear until it is shaken to distribute the mycelium. But a few days later, the contaminants will bloom, taking advantage of the lack of peroxide protection in the newly multiplied zones of mycelial growth. This problem can be prevented by cleaning reusable containers carefully, both inside and out, before use, and wiping down the outsides of the containers with rubbing alcohol after the spawn has been inoculated.

Here's how I make soft white wheat spawn:

1) I weigh out 7 oz of grain into a 26 oz jar.

2) I then add an excess of hot tap water and a tiny amount of baking soda to offset the acidity of my tap water.

3) Next, I steep the grain at near-boiling temperatures for an hour or two to swell the kernels, draining off the excess water when the grain has about doubled in volume.

4) Finally, I sterilize the jar of grain in a pressure cooker for an hour. The exact length of time you use will depend on your grain and pressure cooker.

5) When the jar has cooled, I add 10 mls 3% hydrogen peroxide (or 20 mls peroxide for every 16 oz grain initially added), then shake well to coat the grain.

One grower adds food color to his peroxide, so that he can see whether he's done a thorough job of distributing the peroxide over the grain. (If your grain clumps significantly, it will be difficult to coat the kernels completely, so take care to adjust your water content, and don’t soak or cook your grain too long). The final peroxide concentration is high, about 0.15%, but mushroom mycelium still grows well, if somewhat more slowly than without added peroxide. (If you make your spawn by adding a measured amount of water, remember to subtract the volume of peroxide

from the water you add, to achieve the correct moisture content). You may well be able to get away with adding less peroxide, but if you add less than 20 mls 3% solution for each 16 oz of grain, you will most likely need to dilute your peroxide into a larger volume of sterile water before addition, to assure thorough coating of the grain by the solution. On the other side, you can add up to 40 mls peroxide without seriously affecting mycelial growth in most cases.

Spawn containers

I grow my spawn in 26 oz pasta sauce jars, since I can get these readily. They have one-piece lids. Quart canning jars will work just as well, especially if you have one-piece lids, but a two-piece lid can be serviceable if you put a slightly oversize cardboard disk inside the lid, so that it holds the top of the lid within the band. Be sure to keep the inside of the lids clean for each use, as well as the lid threads on the jars. Traces of old medium around the mouth of the jar or in the lid can cause major problems. Rusty spots on the insides of the lids can also catch such traces of medium and provide a place for microbial growth.

Note that peroxide addition makes unnecessary the use of lids fitted with microporous filters as were traditionally required. However, the jar lids are a vulnerable area, even with peroxide added to the medium, since you will be shaking the jars to distribute mycelium, and the shaking can bring airborne mold spores that have diffused inside the lid (or bits of mold that have grown in the crevices of a poorly cleaned lid) into contact with the mycelium (which itself is unprotected). To compensate for lid vulnerability, I do the following:

1) I prepare a set of thin cardboard disks cut to fit inside my jar lids (cereal box cardboard works well; just trace a circle around the lid onto the cardboard with a pen, then cut slightly inside the traced circle with a scissors).

2) For "10 minute spawn," I mix the ingredients with a separate lid, then I put the lids with cardboard disks in place just before steaming.

3) As the spawn cools, I open the lids and wet the cardboard disks with 3% peroxide by freepouring a couple milliliters into each lid. The peroxide-moistened disks then form a barrier to airborne contaminant entry.

For grain spawn or other spawn that requires pressure sterilization, I wrap the disk-containing lids individually in aluminum foil, sterilizing them separately from the jars of spawn, which I sterilize with temporary lids in place. Then, after adding peroxide to the sterilized spawn substrate and shaking it in, I remove the temporary lid and put in place one of the sterile lids

with a cardboard disk. I then wet the disk with 3% peroxide solution.

Inoculating spawn

Inoculating jars of "Ten Minute Spawn"

by the agar chunk method

Sterile containers of spawn medium can be inoculated in a couple of ways. You can cut chunks of mycelium out of agar cultures with a sterile scalpel and drop the chunks into the container. (If you do this, first tip the jar or bag to make the substrate slope to one side, so you can get the chunks of agar a ways down into the substrate, but still at the side of the container where you can check for growth). Or you can shake the container after adding the chunks. I prefer not to shake the container, because the chunks often end up sticking above the medium, unprotected by peroxide, and they are difficult to dislodge by further shaking. With peroxide addition as well, there is no clear benefit from shaking the chunks with the substrate. The small fragments of mycelium that are broken off this way seem to be too small to effectively recover and grow in the presence of peroxide at the high concentration used in spawn medium. Therefore, I drop the agar chunks (three pieces has been adequate for slow growing strains) down into the substrate and close the container. With sawdust spawn of H. erinaceus, I then tap the jar on my counter to pack down the spawn medium around the agar chunks, since the organism seems to prefer a dense, closely-packed substrate.

Note that peroxide-treated spawn medium should only be inoculated with peroxide-adapted mycelium, that is, mycelium that has grown out on peroxide-containing agar. Otherwise, the unadapted mycelium may die off or take a very long time to initiate new growth when confronted with the relatively high concentration of peroxide I have suggested for spawn making. (Peroxide-treated bulk substrate, however, contains a much lower concentration of peroxide, so it can safely be inoculated with spawn that has not been adapted to peroxide.)

My original procedure was to put my inoculated jars inside fresh plastic food storage bags tied closed. (I would do this immediately after wiping down the jars with rubbing alcohol). I used the plastic bags to provide a still-air environment, and to keep out stray fungus gnats. (The bags can be reused, provided they are still clean). Lately, however, I have been incubating the spawn jars without enclosing them in food storage bags, and this appears to work almost as well.

Finally, I make sure the jars are sealed completely and I let the mycelium grow out from the agar for several days. Decomposition of the added peroxide provides oxygen to support mycelial growth up to this stage, and carbon dioxide levels are not yet very high. When I have a halo of growth

about a centimeter wide, I then shake the spawn, and this now results in new growth appearing at many new places in the medium within a few days. (Don’t wait too long to shake your spawn, as the amount of peroxide left to protect the mycelium steadily declines as the halo of mycelial growth from the agar chunks grows wider). I used to loosen the lid to the jar slightly after shaking, to allow gas exchange, but I now find this to be unnecessary. The cardboard disk evidently allows enough gas exchange even with the lid tightened down.

The spawn is ready to use when the mycelium has grown through the spawn medium lightly but completely. I usually wait until the mycelium has started to extend about a half a centimeter or more above the top surface of the medium before I use a spawn jar for inoculation.

If you are using spawn bags, rather than jars, the procedure is essentially the same. You won’t have to worry about contaminants entering the bags as they cool--any that do enter will be killed by the added peroxide.

What about using peroxide to make liquid cultures? I have not pursued this possibility, for two reasons. The first is that any method of inoculating a liquid culture is likely to require blenderizing the inoculum (or in some other way breaking up the mycelium), which releases significant quantities of peroxide-decomposing enzymes into the medium upon inoculation. The second reason is that, even assuming the first problem could be overcome, I would still expect the peroxide concentration to decline rapidly in a liquid culture as the intact fungal material with its internal peroxide-decomposing enzymes circulates throughout the liquid. (With solid substrates, the mycelium is confined to one area, and the peroxide concentration in the remaining substrate stays at a desirable level). The decline in peroxide could be compensated for by .regular addition of fresh peroxide, but this might require a method of measuring peroxide concentration in very dilute solutions.

Colonization of bulk substrate

Colonization of bulk fruiting substrates is the third stage of mushroom growing, leading directly to the production of edible mushrooms.

Because hydrogen peroxide solution is so cheap, it is economically feasible to add enough peroxide solution to fruiting substrates to help keep them free of contaminants. And from a technical standpoint, doing so can make it possible to grow a number of wood decomposing mushrooms without having to autoclave or pressure-cook the substrate. For the procedures in this

volume, however, the substrate chosen has to be one that is devoid of peroxide-decomposing enzymes. Peroxide will provide little or no benefit with substrates that still have a great deal of biological activity, such as compost, or pasteurized straw, or fresh wood chips that have been treated with boiling water.

The first material I found to be ideal for use with peroxide was pellet fuel for wood pellet stoves. This substrate comes previously heat-treated, so it will not cause peroxide to decompose, even without autoclaving. As a result, pellet fuel can be conveniently pasteurized for use as a fruiting substrate by adding boiling water, which becomes part of the process of bringing the substrate to the proper moisture content. (When you add boiling water to fuel pellets, they turn back into the sawdust they were originally made from). Hardwood fuel pellets are generally the best bet for most wood-decomposing mushrooms, but pellets made primarily from Douglas fir may work for your strains too. (I suspect that the heat and pressure used in creating the pellet fuel may break down some of the mushroom-inhibiting resins in the fir). Look for a brand of pellets that does not have any additives--that is, plastic binders. Most do not.

Another substrate that I have used with peroxide is recycled pelletized paper fiber. In my area, this is sold as Crown Animal Bedding™, and as Good Mews™ Cat Litter. These products have been sanitized by a double heat treatment (according to the promotional material). The pellets are still about 30% moisture before adding any water. As with the pellet fuel, the material has no residual peroxide-decomposing activity. The drawback here is cost, as the animal bedding is usually priced about three times higher than pellet fuel on a dry weight basis.

If no pellet fuel or paper fiber pellets are available in your part of the world, you should plan to use one of the procedures presented in Volume II for your substrate preparation. Those procedures allow a greater variety of possible materials as substrates, including some that contain peroxide-decomposing enzymes. Indeed, one of the only materials that will NOT work with any of my peroxide procedures is raw sawdust, that is, the sawdust produced by the milling of raw logs. If you have another substrate you'd like to use with peroxide, say paper waste or cardboard, but you plan to pasteurize it rather than autoclaving, you will need to be sure it is free of peroxide-decomposing enzymes after pasteurization. You can test it simply by putting a small amount of the substrate in a cup and adding some fresh 3% peroxide solution. If nothing happens right away, let it sit for a while. When peroxide-decomposing enzymes are present in the substrate, the mixture will bubble and froth. If the enzymes are all gone, the mixture will look no different from substrate mixed with water.

Recipes for fruiting substrates vary from one mushroom species to the next. For wood-decomposing

mushrooms, most recipes include sawdust (which we will now derive from pellet fuel), at least 1% powdered lime, water sufficient to give a final moisture content of about 60 to 65%, and from 5-20% of the dry weight as some kind of nitrogen rich supplement like rice bran (which provides about 0.1% to 0.4% nitrogen overall).

Higher nitrogen levels in supplemented sawdust generally translate to higher yields of mushrooms, but traditionally, high nitrogen has also translated to greater risk of contamination. With the peroxide method, the danger of contamination may not increase appreciably with higher nitrogen levels. However, to be on the safe side, I seldom raise the nitrogen level above 0.4%.

Wood chips and substrate density

Traditional recipes often call for wood chips, but I have never included them in my substrate since it would require the inconvenience of pressure-cooking the chips separately and adding them to the pasteurized bulk substrate later. Some growers believe wood chips are crucial for good growth of shiitake. I have not found them necessary for H. ulmarius, P. eryngii, or H. erinaceus. However, for H. erinaceus, I have found it beneficial to gently but firmly compress the sawdust in my cultures after inoculation by pressing with my hands on the outside of the bag, removing the air space (but not the absorbed water) in the substrate. This may serve something of the same purpose as adding wood chips, by creating a substrate of greater density. I can well imagine that an organism like H. erinaceus, which can grow happily through woods as dense as walnut and cherry, might prefer a dense substrate and thus do better on compressed rather than fluffy sawdust. In traditional methods without peroxide in the sawdust, it would not have been advisable to compress the substrate, because of the danger of creating anaerobic conditions favorable to deleterious organisms. With peroxide in the substrate, however, decomposition of the peroxide provides a beneficial level of oxygen even in compressed substrate, thus making it possible to provide the density some of the organisms prefer without inducing anaerobiosis.

Preparing supplemented sawdust with peroxide

So here's what to do with the pellet fuel:

1) First, find a container such as a five gallon plastic bucket with a tight-fitting lid, and clean it thoroughly. (For my routine cleaning, I wipe down the inside of the bucket with a scrub sponge and biodegradable dish detergent, then rinse this out.)

2) Next rinse the container and its lid with boiling water -- a teakettle-full should do. From

here on out , you will need to avoid touching the inside of the bucket or the rim.
3) Place the loosely-closed bucket on a scale and scoop in about 8.0 pounds dry weight of pellets if you have oak, or 6.0 to 6.5 pounds if you have a lighter wood like fir (six pounds is roughly a gallon of pellets, if you prefer to measure by volume. I use a one quart glass pot that IÕve boiled some water in on the stove to do my scooping, but it doesn't really need to be pasteurized). You will probably have to make your own adjustments for your local pellet fuel, setting the weight you use according to the amount of sawdust you can fit in your bucket along with your supplements and spawn, while still leaving enough room to mix the contents of the bucket efficiently.
4) If you are using a solid, denatured nitrogen supplement like Sylvan's CG60 or Millichamp 3000, it can be added to the pellet fuel at this stage.
5) Add your lime to the pellet fuel. I use powdered oyster shell lime, but before I use it, I bake my supply at 400 degrees F for a couple of hours to eliminate any peroxide-decomposing enzymes resulting from microbial growth on the shells. Crushed limestone is also a good choice if you can get it. Do not use dolomite lime, which contains magnesium that can inhibit mushroom development. For mushrooms grown on oak pellet fuel, I use 2 oz of lime, or half that much for lighter pellet fuel such as cottonwood.

6) Boil in a covered pot half the amount of water you want to add to the pellets. (Your water should be clear and free of any obvious particulates. In some cases this may necessitate filtering).. For this step, I boil about 3.5 quarts (or about 3.5 liters) for 9.0 lbs of the oak fuel pellets. (If you are using a soluble nitrogen supplement such as artificial fertilizer, it can be added to the water before boiling). Fir pellet fuel sawdust is less dense, so I use only about 6.5 lbs of it, boiling 3 quarts of water. You may want to experiment with different moisture contents for the species you are growing. One advantage of adding peroxide to your cultures is that you can add more water than you could otherwise, without developing anaerobic areas in your substrate that might lead to contamination. However, the pellet fuel sawdust tends to clump as more water is incorporated, which makes it harder to pour into the bags later on without spilling).
7) When the water has boiled for a minute, set the lid of the bucket carefully to one side and pour the boiling water over the substrate. Seal the lid and mix the substrate by turning the bucket for a couple of minutes to distribute the water.
8) Boil in a separate covered pot the other half of the water you want to add to the pellets

(e.g., another 3.5 quarts/liters of water). When the water has boiled for a minute, turn off the heat and set this pot of water aside to cool with its cover in place. You'll use this water to add peroxide later.

9) Set the bucket of substrate aside to cool, with the lid in place. Cooling usually takes several hours. The bottom of the bucket can still be somewhat warm to the touch at the time of peroxide addition.

10) With a boiling-water-rinsed measuring cup, add about 1/2 cup of 3% peroxide solution to the pot of cooled, boiled water you've set aside.

11) Pour the peroxide mixture into the cooled bucket of substrate and mix thoroughly by turning the bucket. This gives a final peroxide concentration of about 0.03%, or a one to one hundred dilution.

12) Let the substrate finish cooling to room temperature. It is now ready to use.

Perhaps you are wondering at this point whether this procedure can be simplified along the lines of the Ten Minute Spawn procedure. If the peroxide concentration were raised to compensate for decomposition in the hot substrate, maybe peroxide could be added at the beginning of the procedure with all of the water. This may indeed prove possible with a high enough initial peroxide concentration. However, the substrate takes more than twice as long to cool when all of the moisture is added initially as boiling water. Under these conditions, I suspect the peroxide will have a difficult time surviving the exposure to heat, even if the initial concentration is raised several fold.

.

Nitrogen supplements for bulk substrate

If you are using traditional nitrogen supplements like millet or rice bran, you will have to pressure cook them. While still hot, the sterilized supplement gets poured into the cooling pasteurized substrate. Be careful to wipe drips off the outsides of the jars before pouring.

Most of the traditional nitrogen supplements for mushroom culture require pressure cooking to eliminate the endogenous peroxide-decomposing enzymes before pasteurization. (These enzymes are remarkably stable, and standard pasteurization procedures are generally not enough to inactivate them, even with delicate supplements like bran). However, as I discussed in the section on making sawdust spawn, I have now found a few supplements that are free of enzymes and so can be added without pressure sterilization, in this case mixed in with the wood pellets. Two of these are commercially manufactured nitrogen supplements already in use in the Agaricus mushroom industry

(Sylvan’s Millichamp 3000 and CG 60). They contain denatured soy protein and corn gluten, respectively, and evidently the denaturing process destroys the peroxide-decomposing enzymes. These supplements are an excellent value, but home hobbyists may have difficulty obtaining them. Also, care must be taken to keep them from spoiling in storage, especially with Millichamp 3000. Some more expensive forms of processed protein are more readily available, such as Texturized Vegetable Protein, powdered soy milk, or powdered cows milk.

Another type of supplement that can be used without pressure sterilization is simply chemical fertilizer, such as a standard 20-20-20 fertilizer. Since these fertilizers do not come from living organisms, they contain no peroxide-decomposing enzymes. Nevertheless, for the most part the nutrients they contain can be utilized by mushroom mycelium after a period of adaptation. If you are going to try this method of supplementation, I recommend that you prepare sawdust spawn using the same supplement, so that the adaptation period will have already taken place by the time you inoculate your bulk substrate. Also, you will get a chance to see how the particular fertilizer you have chosen will work for the organism you are growing. Fertilizer formulations vary quite a bit, even with the same NPK rating, so it is probably advisable to test your selected fertilizer with a small culture before going on to bulk substrate.

Urea is a common source of nitrogen in the formulations for chemical fertilizers, and it probably can also be used by itself as a supplement without pressure sterilization.

If you want something more "organic" than artificial fertilizer (and there is good reason to avoid dependence on substances which require energy from petroleum for their manufacture), human urine and animal urine can also serve as supplements that don’t need pressure cooking. However, they must be kept relatively free of micro-organisms until use. Addition of peroxide provides one way to do this.

Calculating how much supplement to add

How do you calculate how much of the various supplements to use? Calculations are only approximate, and you will ultimately need to make decisions based on your actual yield of mushrooms at various levels of supplementation. But you can get an idea of whatyou'll need by consulting Stamets's Growing Gourmet and Medicinal Mushrooms, where the appendices reveal that rice bran has an NPK rating of roughly 2-1.3-1. So, if substrate would ordinarily get supplemented with 5 to 20% rice bran, which Stamets suggests, then a 20-20-20 fertilizer, which has 10 times as much nitrogen as rice bran, would be added at one tenth the rate of rice bran, or

0.5 to 2% of the dry weight of substrate. If you were adding one pound of rice bran to a bucket of pellet fuel, then you would add 1.6 oz of 20-20-20 fertilizer instead, that is, one tenth of a pound. With the commercial supplements, you will need to find out from the manufacturer what percentage nitrogen the material contains, and divide that number into 2.0 to learn what fraction of the amount of rice bran you would add. Sylvan's Millichamp 3000, made from soy, is about 7.3% nitrogen, so it will need to be used at about one quarter the rate of rice bran.

You can also directly calculate the amount of the material needed to give 0.1% to 0.4% nitrogen in the final substrate, without reference to the amount of rice bran used by Stamets:

1) Divide the percent nitrogen in the supplement by the final percent nitrogen desired in the substrate.

2) Divide the previous number into the total weight of substrate to be supplemented to get the weight of supplement to be added.

Thus, to get 0.2% final nitrogen (which requires a supplementation rate of roughly 10% of the dry weight of substrate with rice bran), how much soy flour would we need to add? If the soy flour is 7.6% nitrogen, 7.6 divided by 0.2 gives 38. If the total weight of substrate is 6.5 pounds, then

6. divided by 38 gives 0.17 pounds, or 2.72 oz soy flour to be added.

A note on measuring pH of substrate

I use ColorpHast strips with a pH 4 to 10 range to measure the pH of all my media and substrates, aiming for a pH at make-up in the 6-7 range in most cases. ColorpHast strips are inexpensive and convenient, and with a three-color comparison, I am usually confident of my reading. However, it is not a good idea to try to measure the pH of medium with a color indicator strip once peroxide has been added, as the peroxide may change the chemistry of the indicator. With agar cultures and spawn, you can easily measure the pH before sterilization. With pellet fuel, use a small scoop such as a measuring cup (one that has been rinsed with boiling water) to take a small amount of substrate out of the bucket after adding and mixing in the boiling water plus lime. You can then use your color indicator strips to measure the pH of the removed substrate. Be aware, however, that the reading will only give you a relative idea of the pH eventually experienced by the mushroom mycelium if you have added granular lime which dissolves only very slowly. Addition of Mason's lime (CaOH, available in large sacks as "builders’ lime" from construction supply such as granular lime provides. Then you will simply have to calibrate the amount of lime you used against the ultimate yield of mushrooms to determine the optimal dose.

Culture containers

Traditionally, sawdust cultures have been grown in special plastic bags with microporous filter patches, to allow gas exchange without letting contaminants gain entry. With peroxide in your fruiting substrate, however, you should be able to use ordinary trash bags (at a savings of $.50-$.80 per bag) to grow your mushrooms. Evidently the process used to produce trash bags pasteurizes them to the point that they do not harbor significant live-organism contamination. If you do use plastic trash bags, I recommend using the kind that are made from high density plastic, 0.5 mil thickness or less. These bags are thin enough that oxygen can diffuse through them, so that the cultures can be grown to maturity with the bags sealed closed by twist ties. Also, the thicker, softer bags are apparently made from PVC, which can leave estrogenic residues in the mushroom cultures, and certain of these softer bags are impregnated with fungicides. Unless you use traditional gussetted mushroom bags, you will need to put your bags inside containers of an appropriate size to provide a form for the substrate. Small boxes which will hold 5-6 pounds of substrate can often be scavenged from health food stores or the like, or you can buy plastic containers such as nursery pots.

Filling a plastic trashbag, propped insidea box, with pellet fuel substrate

Disposable bags create considerable waste for the landfill. An alternative is to use plastic buckets with lids, 2 or 3 gallon in size, preferably HDPE plastic, recycling number 2. (Five gallon buckets are easier to come by, but they are a little too big for your average sawdust culture). These can be cleaned with detergent and reused after rinsing with boiling water. . If the lids are left slightly loose during the spawn run to allow gas exchange, the buckets are excellent culture vessels for mushrooms species that fruit vertically, such as P. eryngii. H. erinaceus will also grow in them if you open the bucket and turn it on its side when fruiting time comes. (I fill the bucket only about a third to half way full with substrate, so the upper part of the bucket provides a moisture barrier). H. ulmarius would be a little cramped for space in one of these buckets, unless you were to fill substrate nearly to the top, so that the mushroom clusters could grow out above the rim. To get a second flush, then, you would need to take the round block out of the bucket and turn it upside down, since H. ulmarius doesn’t like to fruit from the same surface twice.

Inoculating Supplemented Sawdust

I prepare spawn for inoculation in the traditional way:

1) The day before I want to use the spawn, I break up the spawn by whacking the jar against something hard protected by something soft.

2) When the particles are separated, I put the jar back on my spawn shelf and incubate overnight to give the mycelium a chance to put out some new growth. This makes a considerable difference in how fast the mycelium will surge into the new substrate. If I am working with grain spawn, it also gives me a chance to see bacterial contamination in the form of "wet" or greasy looking grain kernels that havenÕt acquired a new fuzz of mycelium. When your spawn has been grown using peroxide, the presence of two or three wet kernels will probably not interfere with the subsequent success of your colonization of bulk substrate, since the bacteria that are able to survive peroxide exposure are generally fairly benign organisms when present in small quantities. However, if you have quite a few more wet kernels than two or three, the bacteria will likely slow the colonization of bulk substrate substantially, which then may give a chance for mold to gain entry. So you will probably want to discard spawn with any significant quantity of wet kernels.

3) I inoculate the pellet fuel sawdust by breaking up the spawn briefly, then pouring it directly into the 5 gallon bucket with the substrate. I close the lid and mix everything together by rotating the bucket. Inoculating a bucket of pellet fuel substrate with spawn

4) I pour the mix into bags. Each bag gets opened up and set inside of a box of the appropriate size to receive the substrate.

5) When I have filled the bag to the capacity of the box, I close the lid on the remaining inoculated substrate, and taking care not to touch the inside surface of the bag, I shift the bag a bit to fill any gaps, then twist the mouth of the bag closed and seal it with a twist tie.

6) Lastly, I compress the sawdust by pressing down on the bag, gently but firmly. I find that this speeds growth of some cultures, especially with a light sawdust like fir or cottonwood.

After labeling, the box is ready to incubate, and from here on out, I follow standard mushroomgrowing procedures. You can use the resulting blocks of mycelium either directly for fruiting mushrooms, or the blocks can serve as spawn for inoculating logs or beds of fresh wood chips outdoors.

Mushroom Formation

For most commonly cultivated mushroom species, mushroom formation begins soon after the cultures are shifted to cooler temperatures, given more light, and given more fresh air, provided the substrate has been thoroughly colonized. There is not much need for hydrogen peroxide during this phase, since the mycelium is well established.

The precise procedures for inducing mushroom formation differ from one species to another, and it is beyond the scope of this manual to review them all, but I will give guidelines for my favorite species. Two of the mushroom species most familiar to me are also among the easiest to fruit: Hypsizygus ulmarius (the White Elm mushroom) and Hericium erinaceus (the Lyon's Mane or Pom Pom mushroom). Many oyster mushroom species follow similar procedures to that required by H. ulmarius. The other species most familiar to me, Pleurotus eryngii (King Oyster) and Agaricus subrufescens (almond mushroom) follow a different fruiting pattern. Shiitake follows yet another.

Most of the "easy" mushroom species are ready to fruit when the bulk substrate is thoroughly grown through. Often the blocks look white at this time, rather than the original brown of the substrate. How long it takes for a culture to reach maturity depends on the organism, the substrate, and the incubation temperature. Hericium can take as little as 2-3 weeks to form small white, globular fruiting initials on the upper surface of the block, but I like to wait until a month has elapsed before opening the bags. H. ulmarius takes about 5 weeks on fir sawdust or straw and six weeks on oak sawdust (at ordinary room temperature), after which small clusters of pinhead primordia will begin to form spontaneously. By cutting an "X" or a single slit with a clean knife through the bag on the side of the block, H. ulmarius and various Oyster mushroom species will usually form primordia at the site of the cut within a week or two, and mushrooms will soon develop. Provide mist spray when the mushrooms are about an inch high.

Hericium will also form mushrooms at the site of a cut in the bag, but in the winter I find it easier to grow large fruiting bodies by allowing the organism to fruit inside the bag. Simply turn the block on its side and open the bag a bit, allowing air exchange but still providing a moisture barrier. Fruiting bodies will form at random from the fruiting initials that have already developed. If you grow only a few blocks of mushrooms, ventilation will not be much of an issue. But with more blocks, the need for ventilation to remove carbon dioxide increases. If your mushrooms are not getting enough air as they develop, they will become deformed. H. ulmarius and other oyster mushrooms, for instance, will grow long stalks and irregular caps when the carbon dioxide concentration is too high. When you have enough blocks to need a fan, then an automatic misting system can• be far behind.

Note that if you decide to grow H. ulmarius or another oyster species in your home, you will

probably need to take precautions to protect yourself from the tremendous amount of spores produced by these organisms. Harvesting the mushrooms when young can help keep the spore load down. Covering the fruiting cultures with Reemay™ or other row cover material will keep the bulk of the spores within the covering, while allowing gas exchange sufficient for fruiting. However, if you or someone in your family is sensitive to the spores, you may need to acquire an air purifier to eliminate the spores from the air in your living space, or else keep the cultures in an out-building. The almond mushroom, the white button mushroom, as well as King Stropharia and Shaggy Mane, and sometimes King Oyster all need something called a casing layer applied to the mushroom culture to stimulate fruiting body formation. Casing is a mixture designed to imitate a moist, friable, loamy soil. It contains microorganisms that promote mushroom formation, and it provides a moisture reservoir for mushroom growth. It generally contains little available nutrition for the growth of mushroom mycelium, and this feature also sends a signal to the mushroom culture to begin mushroom formation. Most casing contains peat moss, and a simple formula I have used for almond mushrooms simply calls for one part peat mixed with one part garden soil, plus a handful of gypsum (calcium sulfate) for two or three gallons of mixture, all moistened until damp but not clumpy. The casing is applied to the top of the mushroom culture to a depth of two inches at the most. Be careful not to tamp it down, as the porous structure is essential for encouraging formation of mushroom primordia. Peat bogs are an endangered habitat worldwide, so we need to find alternatives to the use of peat in casing. Growers in different parts of the world have begun to devise alternatives, as one can discover by searching the US patent database (see http://www.uspto.gov/). In some cases, soil alone may suffice as a casing, or soil plus vermiculite. Vermiculite by itself is a possible alternative (although it will not supply microorganisms). Almond mushrooms and King Oysters do not absolutely require a casing (the casing does tend to accelerate primordia formation with P. eryngii, however), so manipulation of conditions may lead to good fruitings without it for these organisms. If you do apply a casing, you will then need to wait a week or two for the mycelium to grow up into the casing before mushrooms will begin to form. With the almond mushrooms, this is the time to warm up the cultures (I put my apple-box cultures of almond mushroom on an electric "cat warmer" to be certain they are warm enough). It is also the time to sprinkle the casing lightly with water every couple of days to keep it moist. (With P. eryngii, warming and sprinkling are not necessary). Mushrooms usually begin to form a few days after the mycelium begins to reach the surface of the casing.

Seasonal planning

I you are only growing a few mushrooms, and you have a cool, insulated indoor space such as a lighted basement, you will probably be able to grow your favorite mushrooms all year. However, if your are growing outdoors, or you are growing a lot of mushrooms indoors (so that you need ventilation from outdoors), you may need to plan ahead to have the appropriate mushroom cultures ready in the right season. I fruit my mushrooms in a basement with open windows and a fan to bring in fresh air, so the mushroom growing gets harder in the hottest part of the summer and the coldest part of the winter. Closing the windows is not an option, as carbon dioxide will build up and inhibit mushroom formation. Heating or cooling the incoming air is certainly possible, but it runs up the energy bill too much for my taste. So all my mushrooms do best in fall and spring.
In the coldest part of the winter, both the temperature and the light levels fall. All the mushrooms take longer to finish colonizing the bulk substrate. P. eryngii fruits with difficulty, and H. ulmarius grows very slowly, producing longer stalks (and deformed caps, if the light and temperature levels are too low). H. erinaceus also grows slowly at this time, but this species still produces normal, but small, fruiting bodies even in very cold weather. Agaricus subrufescens needs warmth, but paradoxically it makes a good indoor mushroom for winter, because it will fruit in heated room and it doesn't need as much air or light as the other mushrooms.
In the hot parts of summer, there is plenty of light, but it can be a problem to initiate fruiting at the higher temperatures. Keeping humidity up can be difficult, too. However, I have had H. erinaceus blocks fruit on a dry compost pile in 90 degree weather; evidently the fresh air initiated fruiting in that case, since my indoor H. erinaceus blocks refused to fruit until the temperature came down substantially. A. subrufescens likes warm weather and tends to fruit as the peak temperatures pass. Ganoderma lucidum also prefers warm weather, as does Stropharia.

Outdoor growing vs. indoor growing

I used to grow almost all of my mushrooms indoors. This allowed me to grow them year 'round because of the more moderate temperature, and it also saved me from having to deal with slugs and snails, which love mushrooms and grow in great numbers in my area. (Actually, I still get a few slugs that manage to climb in my windows, travel down the cement basement wall and across the cement floor, and eventually enter my fruiting cultures). Deer may also eat mushrooms, and fungus gnats can be a problem in outdoor mushroom patches. So in the past, I have always recommended indoor growing. But outdoor growing has its advantages, too. For one thing, mushrooms fruited

outdoors have a distinctly superior flavor to mushrooms grown indoors. Also, mushroom formation can be more robust in the fresh air of the outdoors. There's more physical space for crops. And there is little problem with breathing mushroom spores. Fungus gnats can be reduced somewhat by covering cultures with a fine light fabric such as Reemay(TM) or other row cover materials. And if you live in a temperate region on the seacoast, you may be able to grow mushrooms outdoors all year 'round. So if you think you can keep all the pests away, go ahead and fruit your cultures outside. You will just need a shaded area that can be kept damp.

Harvesting

Knowing when to harvest mushrooms is largely a matter of knowing how large they grow and what changes they go through as they mature. With P. eryngii and H. ulmarius, the uncurling of the margin of the cap is usually a sign that the mushroom has reached maturity, but you will need to correlate this change with the size of the mushroom to be sure. With A. subrufescens, the cap opens and the gills begin to turn reddish in color. With H. erinaceus, small "icicles" form and the mushroom softens. Most mushrooms are said to be tastier if harvested before they start releasing many spores, although they may still gain more mass if left to grow further. It is certainly true that H. ulmarius is tastier when young, but I haven’t personally made taste comparisons with the other mushroom species I grow.

Trouble Shooting

Despite my use of hydrogen peroxide to protect my mushroom cultures, there have been many occasions when things did not go as I had planned and contamination appeared. Each time, I had to track down the problem and correct my procedure, and each timed I was relieved to learn that the use of peroxide itself was not flawed. The procedures I have described here to the best of my knowledge incorporate everything I have learned from my mistakes and should cover the key points required to produce contamination-free mushrooms with peroxide-based culture. Nevertheless, troubleshooting is an unavoidable part of mushroom culture, and you will have to do it sooner or later.

I always find it discouraging to read through lists of things that can go wrong, so instead, I have created a list of questions that draw attention to different aspects of the culture process

for the purposes of troubleshooting contamination problems.

If you are adding peroxide, and you still suffer significant contamination, you might ask yourself some of the following questions:

Is the concentration of peroxide in your stock solution what it should be? Has it been over a month since you measured it?

Is your pressure-cooking equipment functioning properly?

Is steam able to enter your jars and equilibrate (are the lids loose enough? If pressure cooking, do you allow five minutes for steam to equilibrate before putting on the pressure regulator?)

Is your stove element (if electric) heating consistently?

Are you cooking at a high enough temperature and for a long enough time to eliminate resident contaminants and, if necessary, peroxide-decomposing enzymes?

Is your substrate moist enough for steam to penetrate?

Has your substrate or supplement spoiled before use?

Is your peroxide getting distributed evenly throughout the medium?

Is your pH reading accurate? (Peroxide is apparently most stable around neutral pH).

If your fruiting substrate is getting contaminated, is your spawn clean?

If your spawn is getting contaminated, is your inoculum clean?

If you are free-pouring diluted peroxide into your cultures, are there drips that run over unsterilized surfaces before falling into the culture?

Are your petri dishes free of traces of old medium?

If your agar plates are getting contaminated, is the contamination on the surface or within the agar? If it is on the surface, look for a source of contamination external to the agar medium; if it is in the agar, contamination is getting in before or during pouring.

Are you letting your medium or substrate cool sufficiently before adding peroxide?

Is your water clean and free of particulates?

Have you overlooked some source of unsterilized or unpasteurized material that can get into your cultures?

Are you mushrooms getting enough light (but not direct sunlight), fresh air, and humidity to grow to a good size?

Conclusion

As I reach the end of this manuscript, I am forced to pause for a moment of self examination. I called this volume **Growing Mushrooms** the **Easy** Way, and now I have to ask myself whether I wasn't indulging in just a bit of self-serving exaggeration when I chose that name. After all, there are still far more ways for things to go wrong in mushroom cultivation than for them to go right. And I sometimes think it is a wonder indeed that we ever get any of these organisms to respond to our coaxing and produce their delectable fruiting bodies. Well, it is a wonder. And even with peroxide maintenance of mushroom cultures, the process is far from fool-proof. But I feel gratified that the procedures described here do make it possible for hobbyists with at least a minimal degree of comfort with sterile technique to perform all the steps of gourmet mushroom growing and mushroom culture in an ordinary household, more easily than ever before, with no more special equipment needed for contaminant control than a steaming pot and a measuring pipette. And with the stress taken off of battling contaminants, home growers should be freed at last to focus on the thing that attracts us all to mushroom growing in the first place, the quest for ever more of those beautiful and delicious fungi.

About the Author

Rush Wayne holds a Masters degree in Biochemistry and Molecular Biology from Harvard University and a Ph.D. in Biochemistry from the University of California at Berkeley. He was first exposed to the elements of mushroom growing during his graduate work in the 1970's but did not begin **growing mushrooms** in earnest until he began to implement the innovations contained in this manual in 1993. Instructions for his peroxide method of **growing mushrooms** are now in the hands of mushroom growers in over 75 countries around the world.

Growing Mushrooms the Easy Way

Home Mushroom Cultivation

with Hydrogen Peroxide

Volume II

by R. Rush Wayne, Ph.D.

CONTENTS

Introduction

I've written this second volume of my manual, **Growing Mushrooms** the **Easy** Way, both to fill in some of the gaps in the first volume, and to introduce some new ideas for saving time, effort, and money in the process of mushroom cultivation. As in the first volume, most of the methods in this second volume have been designed primarily for small scale and home cultivation. But I designed the first two methods for preparing bulk substrate specifically for growers who want to work at commercial scales. And nearly all of the remaining techniques presented here could also prove useful in a commercial context. The procedures are broadly organized within the volume according to the stage of mushroom cultivation they apply to. So, the techniques relating to maintaining cultures and germinating spores come first, then a method for preparing spawn, finishing with the procedures for preparing bulk substrate.

Although the procedures in this volume largely stand on their own for growers familiar with the peroxide method, if you are a newcomer, you'll want to refer to the first volume of **Growing Mushrooms** the **Easy** Way for essential background information on mushroom growing generally and on the use of hydrogen peroxide in mushroom culture in particular. The peroxide-based methods presented in this volume are all my original inventions. In general, the non-peroxide methods have been worked out by others and have made there way into the public domain. I am presenting them here because of their obvious value to mushroom growers who use the peroxide method.
Enough said--let's get started!

Acquiring, Storing and Maintaining Mushroom Cultures

Using slants instead of agar plates

It has long been the standard procedure in mycology to grow mushroom tissue cultures in Petri dishes filled with agar medium. But as more and more people seek to cultivate mushrooms without sterile laboratories, this standard has come into question. Perhaps the biggest drawback of agar Petri dish culture for mushrooms is that the dishes have such a large exposed surface area, and they are incubated for so long, that the chance of picking up airborne contamination is high. Even with peroxide in the agar, mold colonies will occasionally appear at the edges of plates, especially after the mycelium has spread over most of the agar, and especially during the "mold season" in the fall. One solution to the problems of agar Petri dish culture is to switch to agar culture in containers that have smaller exposed surface area--for instance, screw cap test tubes (typical dimensions are 19 x 125 mm.). These are relatively **easy** to handle and store, and the mouths of the tubes can be readily held over the flame of an alcohol lamp for a moment on opening and closing, for added security against contaminants. The water in these tubes evaporates much more slowly than in Petri dishes, so the peroxide concentration remains at an effective level for much longer. Even without peroxide, the contamination rate is low. What’s more, slants use less agar than Petri dishes, so they save you money. The downsides are: some mushrooms don’t like the wetter environment in slants (for example, H. ulmarius); you can't monitor the morphology of the mycelium as closely in slants as on agar plates (changes in morphology can indicate contamination or other strain problems); and it can be frustratingly difficult to dig out chunks of mycelium from slants when you want to inoculate other cultures (this depends quite a bit on the kind of mycelial mat a given mushroom lays down).

Making slants

Here's how to make a set of slants:

1. Measure out the ingredients for MYA medium (see below--250 mls. will make about 20 slants or so) into a clean metal can. Squeeze a crimp in the rim of the can to serve later as a pour spout.
2. Put a piece of aluminum foil over the top of the can, and put the can in your pressure cooker with some water. Put your screw cap tubes in at the same time, perhaps sitting in another can.
3. Steam for 10 minutes without the weight in place, then put the weight on, bring the cooker to full pressure, and cook for 10 minutes more.
4. Remove the cooker immediately from the heat and cool. Open the cooker as soon as the pressure has gone down, and take out the can and the tubes to cool. You can set the can in a pan of warm water to speed the cooling, but don't let it start to solidify.
5. Add peroxide (1.5 mls. for 250 mls. of MYA medium) with a pasteurized pipette (i.e., one steeped briefly in boiling water) and mix it in by swirling the can.
6. Finally pour the agar into the tubes, using the pour spout on the can to get the agar into the narrow mouths of the tubes without spilling over the tube threads. Hold the screw cap curled in the little finger of your dominant hand while you do the pouring.
7. Replace each cap and set the tubes in a bowl as you finish each one, so that they cool at a slant. When cool, they are ready to use.

For **easy** reference, here's the recipe that I use for MYA Medium (from Volume I):

12 gms (0.35 oz) agar
12 gms (0.42 oz) light malt powder
1 gm ((0.035 oz) nutritional yeast powder
0.5 gm (0.017 oz) grain flour (I rotate between wheat, rye, corn, rice, oats, and millet)
0.5 gm (0.017 oz) rabbit feed (or other animal feed pellets)
5-7 wood fuel pellets (the number of wood pellets can be increased for those wood-decomposing species that do poorly on agar)
1 liter tap water
(Adjust pH to 6-8 with a bit of baking soda or vinegar)

Making transfers to and from slants

For inoculating slants, and for withdrawing mycelium from them, it helps to have an inoculating loop

made of fairly stiff wire. You can make an inoculating loop for yourself by putting a small loop in the end of a wire, then inserting the other end of the wire in a thin glass tube (1/8 inch, for example). Finally, use a flame to melt the end of the glass tube closed over the wire to hold it in place. The glass tube becomes the handle.

For successful inoculation, you will still need to dig out a chunk of agar plus mycelium and transfer it to the slant. This can be a tricky endeavor with an inoculating loop. (If the chunk is too small, or if you simply scrape mycelium off the surface of the agar, it may not be able to establish itself. But if at first you don't succeed, you can always try again with the same slant, since the peroxide will keep it free of contaminants.) I like to dig the loop into the agar to cut out a piece of the culture, but it can still be hard to catch a good-sized piece on the loop.. Many times I've pulled out such a piece only to have it drop off the loop as I moved to transfer it. But eventually you'll do it. Roll the mouth of the screw cap tube in the flame of your alcohol lamp before and after the transfer.

Cleaning the mycelium with slants

In Volume I of **Growing Mushrooms** the **Easy** Way, I explained how invisible microbial contamination can build up on the mycelium of cultures grown by the peroxide method, and I presented a method for "cleaning" the mycelium of these contaminants by inoculating the bottom of the agar in Petri dish cultures. With slants, the invisible contaminants should build up quite a bit more slowly than with Petri dishes, but they will eventually accumulate to the point where the mycelium needs cleaning. There is, however, no convenient way to inoculate the bottom of the agar, so we have to use a different method. This method consists of pouring a second layer of agar--when it is almost cool enough to solidify--on top of mycelium growing on the first layer of agar. The mycelium then grows up through the second layer, cleaning itself as it goes.

Here's what to do:

1) Prepare and inoculate slants as described above. Allow the mycelium to grow out a bit over the agar.

2) Prepare a small amount of MYA medium by the standard procedure, but use a can with a crimp in the rim for a pour spout (as for making slants above) to hold the medium, and cover it with aluminum foil.

3) After cooking, when the medium has cooled substantially, add peroxide at the usual concentration and mix thoroughly by gentle swirling.

4) When the bottom of the can is barely warm to the touch, light your alcohol lamp. Open a slant, holding the cap in the crook of your little finger, rotate the mouth of the tube over the flame of the

alcohol lamp, and then pour enough of the fresh agar medium into the tube to cover the mycelium completely (you can either cover the mycelium with the tube "slantwise" or with the tube standing upright). The less agar you use, the smaller distance the mycelium will have to grow to reach the surface. Beware! The remaining agar in the can tends to solidify rather suddenly, so put it in a pan of water that is slightly warm.

5) Return the screw cap to the tube.

6) Allow the mycelium to grow up through the new layer of agar.

When taking out mycelium for inoculations, be careful not to dig through the top layer of agar into the lower layer, or you will defeat the purpose of the layering.

Starting with Spores

I usually urge would-be mushroom growers to begin their mushroom explorations with healthy, established tissue cultures of the mushroom species they want to grow, obtained from reputable suppliers of mushroom cultures. Such cultures have been tested and shown to produce good yields of mushrooms under certain reproducible conditions. Unless the culture is subsequently damaged, it will continue producing good yields of mushrooms when you supply the right conditions. And if you lose the culture, you can (in most cases) go back to your supplier and obtain another copy, which will produce mushrooms under the same conditions that worked well for the previous copy. If you start with spores, by contrast, there is no assurance that the mycelial culture you grow from them will fruit in useful quantities, or that the mushrooms produced will have the characteristics of the mushroom the spores came from. You also will not know the optimal growing conditions for the strain you obtain by spore germination until you have tested the strain yourself. And if you lose the culture, you will have to start over again from scratch. Still, there are arguments on the other side. Cultures obtained by spore germination are often quite vigorous. With fast growing species like oyster mushrooms, the differences between the parent mushroom and the spore produced progeny are often small. Spores may cost little or nothing, whereas tissue cultures can be expensive.

Spores can be obtained from dried mushrooms whereas tissue cultures can't. And so on. So it is certainly worthwhile to have a simple method of germinating spores. In previous editions of the peroxide manual, I insisted that spores could not be germinated on peroxide medium. But a couple of people wrote me to say that they had indeed germinated spores in the presence of peroxide. So now I have to acknowledge that yes, it can be done--generally only if you can apply a concentrated dot of millions of spores to the surface of the peroxide medium, but it can be done. Still, I am not convinced it

is a good idea for routine use. The mycelium generated this way could easily be genetically damaged. And as it turns out, it is not especially hard to start clean cultures from spores in the absence of peroxide, even in a non-sterile environment. One good method is to start the spores in screw-cap slants, so the chance of airborne contamination entering is reduced compared to Petri dishes (many thanks to David Sar for letting me know about this approach).

Making slants for spore germination

To make these slants, follow the usual protocol for making MYA medium, with just a couple of changes. The slants will not have peroxide in them, and that means you can

1) melt the agar medium by steaming for 10 minutes, then

2) pour the melted medium into the tubes,

3) close the caps loosely, and pressure cook the tubes with the agar inside them.

4) Finally, let the pressure come down on the cooker, remove the tubes, and let the agar solidify with the tubes in a slanted position. As an alternative, you can sterilize the medium and the tubes separately, then pour the agar into the tubes when the agar is still hot, straight out of the pressure cooker if possible (use thick rubber gloves to handle them). Then let them cool and solidify with the screw caps in place, slightly loose so the pressure equilibrates. To start spores for one kind of mushroom, you'll probably want five or six slants, so you can lose a couple to contamination. Also, they may not all germinate.

Collecting Spores

Here's a way to collect spores:

1) Pressure cook a few Petri dishes for 15 minutes or so in a covered container. Let the dishes cool.

2) Pick a mature mushroom for your source of spores, preferably a clean one that is not likely to be covered with mold spores.

3) Clear a counter someplace in your house where there won't be too many air currents from people walking by. Clean the counter with a sponge and then wipe it down with rubbing alcohol.

4) Open one of your Petri dishes (you can just set the lid, top surface up, next to the dish on the counter) and arrange your mushroom over the dish. If it is big enough, the mushroom can cover the entire bottom half of the Petri dish. Or perhaps you have a cluster that can span the dish. Smaller mushrooms, or mushrooms with odd shapes like morels, can be suspended above the plate by a thread. Use your imagination. Ideally, you don't want the mushroom touching the inside of the Petri dish bottom, and you don't want too much of the Petri dish exposed to airborne contaminants, but you want

the mushroom arranged so its spores can fall into the dish.
5) Depending on the mushroom and the rate of spore fall, go ahead and collect spores until you have a visible coating of spores (this takes about an hour for a copious spore-producers like the oyster mushrooms, but you might need to go overnight for some mushroom species), then close up the Petri dish.

Germinating Spores

Here's one way to germinate spores:

1) Set up and light an alcohol lamp. Have your non-peroxide slants nearby, and your Petri dish of spores.
2) Sterilize an inoculating loop in the hot part of the flame.
3) Open one of the slants (removing the cap with your little finger), roll the mouth in the flame, and plunge the inoculating loop into the agar, trying to pick up a bit of agar on the loop to make it sticky. Withdraw the loop, roll the mouth of the tube in the flame again, and replace the lid on the tube.
4) Open the Petri dish of spores and draw the inoculating loop over the bottom surface of the dish, through the coating of spores.
5) Open the slant again, roll the mouth in the flame, and insert the inoculating loop, this time drawing the loop over the surface of the agar.
6) Withdraw the loop, flame the mouth of the tube and replace the cap tightly.
7) Incubate the inoculated slants. Germination can take anywhere from a few days to a few weeks, depending on the mushroom species.
Eventually, you should see some small white colonies of growth appearing in the slants. You will have to distinguish the colonies of mushroom mycelium from any colonies of contaminants. Some molds will also make colonies that are white at first, but they will turn blue or green as they start to sporulate. Wild yeast and bacteria can make shiny colonies that are light colored, but they will not generally be white, nor will they develop fibrous mycelium. As soon as you are reasonably sure that you have a colony of mushroom mycelium, you should transfer it to peroxide medium. On a peroxide-treated agar plate, you can observe the halo of growth formed by the spreading mycelium. If the mycelium is not homogeneous or it carries contaminants, the halo will likely show sectors that grow at different rates and with different appearances. You can then take mycelium from the best-looking sectors--with good radial or rhizomorphic growth--for transfer to fresh plates. Once you have stable, non-sectoring

mycelium, you are ready to make spawn and test your strain in bulk substrate. And don't forget to make storage cultures!

Ideas Toward Mycelial Culture without Agar

Agar is probably the most expensive ingredient used in mushroom growing. And although it is relatively simple to handle once you've been introduced to it, many beginning mushroom growers undoubtedly avoid getting their own cultures because of their unfamiliarity with agar. So I have been thinking about alternatives. Here I suggest one simple choice that shows promise as an alternative to agar medium--it's gray cardboard disk culture. Gray cardboard is cheap and reasonably available, and as long as it is clean, it will not contain any peroxidedecomposing enzymes. Unlike corrugated cardboard, gray cardboard wets easily and the wet material is soft enough to remove clumps for transfers. It is also a good substrate for the growth of mushroom mycelium, often supporting rapid growth even when very limited amounts of other nutrients are present. And it is a simple matter to add a nutrient solution if you need it. So, instead of going through all the trouble of weighing ingredients for agar medium, melting the agar, cooling slowly, adding peroxide, pouring plates, waiting for them to solidify, then drying them for a couple of days, you can just cut disks of gray cardboard to fit your Petri plates or jars, add a measured amount of water to the disks, and prepare a jar of plain water or a simple nutrient solution. Then, pop the plates and the jar of liquid in the pressure cooker for 10 minutes, cool rapidly, and add peroxide to the jar of liquid. Finally, transfer a measured amount of the solution to the cardboard to give it peroxide protection. The cardboard plates are then ready to use as soon as the solution has soaked in. Where gray cardboard is in short supply, newsprint is a possible substitute, but it has several drawbacks compared to cardboard. For one thing, it can be hard to see the mycelium, particularly if the newsprint is light in color. The mycelium can be as wispy as a spider web when it is growing on newsprint anyway, and to complicate matters it may spread more beneath the surface, out of view, than in plain sight. Moreover, the growth rarely develops in a nice round halo such as one gets on agar, nor does it necessarily progress evenly from one layer of newsprint to the next. Instead, the mycelium can spread somewhat capriciously, apparently influenced by small variations in the conditions it encounters between the layers of the newsprint.

I do not yet know whether mycelium can be repeatedly transferred on plain, unsupplemented cardboard without running into nutrient limitations. I would expect cardboard to be close to devoid of nitrogen, so until further notice, it is probably a good idea to add a nutrient solution to keep your mycelium going on cardboard disks.

How to prepare the plates

Here are the detailed steps for making cardboard plates. Note that you can also use small jars in place of Petri dishes.

1) Measure about 100 mls. of tap water into a small jar.

2) For nutrients, , measure another 100 mls. of tap water into a second jar and add one drop of ordinary soy sauce to the water, and a quarter teaspoon (1.25 mls.) of molasses or light malt powder.

3) Find some gray cardboard, the thicker the better, preferably gray on both sides. Trace a Petri plate onto the cardboard with a pencil and cut out several disks to fit into your plates.

4) Weigh one of your disks and record the weight. Multiply this weight by a factor of 1.3 as a rough guide (you may need to experiment with the amounts here), and add the resulting weight of tap water or nutrient solution to each disk in its Petri plate. (Remember, 1 ounce of water equals 28.35 grams; one gram equals one milliliter.)

Example: Suppose my disks weighed 0.17 ounces each. Multiplying 0.17 by 1.3, I get 0.22 ounces. There are 28.35 grams in an ounce, so 0.22 ounces x 28.35 equals 6.3 grams. That means I'll add 6.3 milliliters of solution to each disk.

5) Close up the disks in the plates, and let the water or nutrient solution soak in.

6) Pressure-sterilize the jar of plain water, and the Petri plates with moistened newsprint disks inside, for 10

minutes at 15 psi (allowing the cooker to equilibrate steam for 10 minutes before putting on the pressure regulator).

7) Cool the cooker, and remove the plates and jar of plain water.

8) When the water has cooled, add 3.3 mls. 3% peroxide to the jar, using a pasteurized pipette, to give you a final concentration of about 0.1% peroxide in sterile water.

9) Add about one third of the initial weight of the cardboard as 0.1% peroxide to each disk. Let the solution soak completely into the disks. They are now ready to use.

You can store and incubate these plates inside plastic food storage bags as I suggest in Volume I for agar plates. But you will probably find that your cardboard disks dry out too quickly. You can keep them moist longer by storing them in a closed container that has some peroxide solution in the bottom. For instance, find a plastic yogurt container or a jar with a mouth wide enough to let Petri dishes pass. Then create a platform to hold your Petri dishes off the bottom of the container, perhaps by putting a smaller jar inside the larger container. Put the Petris on top of the platform. Then add a small quantity

of peroxide solution to the container at the same concentration you use for your plates (roughly 0.018%). Finally, cover the container with a layer of plastic wrap and fix it in place with a rubber band around the mouth of the container (this kind of closure will allow adequate oxygen diffusion). Be careful to set it up so that you cannot knock your Petri dishes off the platform into the water.

Making transfers

Now you are probably wondering how you will remove wedges of cardboard when you want to make a transfer from one of these plates. Well, you won't cut wedges, but here's the trick: you can easily scrape material off the surface of the moist disks using the point of a sterile scalpel. Just draw the scalpel tip firmly sideways across the cardboard a few times in one place. The scrapings can then be transferred to another plate or to a jar of spawn with the scalpel. Corrugated cardboard turns out to be too tough for **easy** removal of material from the surface by scraping in this fashion.

Cleaning the mycelium

As I explained in Volume I and in the section on slants above, invisible contaminants from the air can build up on the surface of mycelium that has been grown on peroxide plates, since the peroxide protects the medium but not the mycelium. The invisible contaminants have to be cleaned off periodically, or else they will proliferate in spawn or fruiting cultures. Mycelium grown on cardboard is no exception. With agar cultures, we cleaned the mycelium with the rather awkward measure of prying the agar disk out of the bottom of the Petri plate into the lid, then inoculating the bottom of the agar, then returning the agar disk to its original place. This forced the mycelium to grow up through the medium, leaving contaminants behind. Although this works, it also increases the failure rate because it is such a tricky maneuver. With cardboard, it is **easy** to inoculate the bottom of the disk: you can just flip the plate upside down, so that the disk falls into the lid and the bottom of the disk is exposed. Then transfer a sample of mycelium to the exposed surface with a flame-sterilized scalpel, close up the plate, and flip it back over. Voila! But as it turns out, the mycelium takes a surprisingly long time to grow through the disk, preferring instead to spread laterally. So rather than waiting for the mycelium to grow to the top, we can simply allow it to spread on the bottom of the disk. As long as it is left undisturbed, the mycelium then will grow entirely under the cover of cardboard, so that it has very little exposure to airborne contaminants. This in itself should keep the mycelium clean, especially if the cardboard disk sits nearly flat on the bottom of the plate. If you routinely inoculate your plates this way, and you I take

material toward the edge of the mycelial halo for your transfers, I expect you should have little problem with accumulation of invisible contaminants. If you have trouble getting your disks to sit flat on the bottom of your Petri plates, you may have better luck by creating a sandwich of cardboard with two sterile peroxide-moistened disks, inoculating the inside of the sandwich, between the disks. The mycelium then will grow entirely within the sandwich, keeping it free of airborne contamination. The dry quality of the cardboard surface on both sides of the mycelium, in addition, should discourage the spread of bacteria and yeast, so that the mycelium can clean itself as it spreads laterally within the sandwich. When you want to get at the protected mycelium inside the sandwich, you pry apart the pieces of cardboard. Because you cannot see how far the mycelium has grown without opening it, you will have to be careful about dating your cultures, so you can be sure you have allowed enough time for the mycelium to grow out before you open the sandwich.

Storage cultures without agar

Freshly inoculated cardboard "sandwiches" can easily be picked up with a pair of tweezers (sterilized in a flame) and transferred to small ziplock plastic bags for storage. After transfer, allow the mycelium to grow out for a week or two. As an alternative, narrow strips of sterilized, moistened cardboard could be inoculated with small chunks of agar culture, then with the help of a flame-sterilized tweezers slipped into sterile screw cap tubes for storage. When it was time to retrieve the culture from storage and grow it out again , a given strip could then be carefully withdrawn and transferred to a sterile Petri dish or a jar, where the mycelium could be more easily scraped from the surface of the cardboard. Yet another choice would be to load some moistened sawdust or paper fiber pellets (broken into small bits after moistening) into screw cap tubes, pressure sterilize for 10 minutes, cool, and inoculate with a bit of agar culture. After the mycelium has grown out, the culture can be put in storage. Then, it should be possible to remove a bit of the culture by means of an inoculating loop or scalpel for transfer to new medium when needed. I have not yet had enough time to determine how well these cultures hold up in long-term storage, but I suspect they will do better than agar cultures, since paper fiber and cardboard more closely resemble natural substrate for mushroom mycelium. If you don't add any nutrient solution, the medium will be quite lean, as is usually recommended for storage cultures; this both encourages dormancy and prevents an accumulation of toxic waste products that the mycelium would produce in a richer medium in long term storage. At the same time, species such as oyster mushrooms that do not do well in wet storage (that is, distilled water or slants) may find the cardboard medium more to their liking, since it has a drier character. In addition, paper fiber and cardboard cultures of

cold-tolerant strains like those of oyster mushrooms can easily be frozen.

Sending cultures in the mail

Just as paper and cardboard cultures can easily be stored in plastic ziplock baggies, so too can they be sent out in the mail this way. Make a sandwich of mycelium between two thin disks of gray cereal-box cardboard that have been sterilized and moistened with peroxide solution. (The colored side of the cardboard faces out, acting to help hold moisture in and keep potential contaminants sealed out.) Then, with a tweezers, pop this sandwich into the smallest ziplock bag you can find, zip it closed, and let it grow out for a few days. Put something heavy on top of the bag like a **book**, to hold the sandwich tightly closed as the mycelium stitches it together. (Instead of a ziplock bag, you can also cut off a corner section of a non-ziplock plastic food storage bag, fold it over neatly, and tape it closed.) Finally, send it off. The recipient at the other end will just need to transfer the mycelium to a fresh plate. To do so, he or she will need to remove the disks to a sterile Petri plate or jar, then pry the sandwich open with a tweezers to get at the mycelium, which has been kept clean and protected inside.
This method of mailing cultures in ordinary envelopes is probably limited to species whose mycelium can tolerate the low temperatures reached in the cargo hold of a jet plane. Warm-growing species such as the almond mushroom (Agaricus subrufescens) may need to be packed in insulated containers to keep them from freezing.

Spawn Preparation
Spawn in plastic bags -- "Eight Minute Spawn"

In **Growing Mushrooms** the **Easy** Way, I presented a procedure for preparing pellet-fuel based spawn quickly and easily using glass jars as containers. This is still my own preferred method for making spawn, but I have worked out variations on this method so that it can be adapted to additional situations.
For instance, perhaps you don’t have a collection of jars, but you can easily get fresh clear plastic bags ("food storage bags"). You can easily use these bags as spawn containers--in fact, they offer certain advantages over jars. For one thing, you don’t need to add paper fiber pellets to the spawn recipe when using bags, which simplifies the formulation process, saves money, and gives you a more finely divided spawn at the same time. (The pellets were there to make it possible to break up the spawn by agitating the jars. With plastic bags, you can break up the spawn by manipulating the bags). For another

thing, air exchange into the plastic bags seems to be greater than that into jars, because oxygen can enter through the plastic but not through glass. This speeds up growth of spawn somewhat in the plastic bags. And, the spawn bags heat and cool more quickly than jars, so the steaming process can be completed even more quickly than before. The bags also save the trouble of preparing cardboard disks to fit in the jar lids, and of cleaning the jars. And finally, the bags allow you to smell the spawn without opening it, because the fragrance escapes through the plastic. This allows you a way to check for purity of the spawn other than just by looking at it. Bacterial and mold contamination will introduce a sour or moldy smell, and each mushroom species has a characteristic fragrance.

On the other side of the equation, the bags create non-biodegradable waste (although they can be washed and reused). The bags also can get pin-holes in them which you can't see. And, they are not especially convenient when you want to remove just a small portion of the spawn at a time (with jars, you can remove the lid, shake out some spawn into a waiting container, then replace the lid). For the latter reason, I still make my spawn masters (which I use for inoculating additional spawn) in jars.

Making the spawn

Here's a recipe for preparing six small bags of Eight Minute spawn, each just the right size to inoculate a 5- gallon bucket of pellet fuel or roughly 6-8 pounds dry weight of substrate.

22 oz (624 gms.) pellet fuel (or other peroxide-compatible material)
4 Tbs. peroxide-compatible nitrogen supplement (see Volume I for options)
0.2 oz (5.7 gms.) hydrated lime or 0.4 oz (11.4 gms.) powdered limestone
0.2 oz (5.7 gms.) gypsum (calcium sulfate)
990 mls. hot tap water
110 mls. 3% hydrogen peroxide

1) Measure the pellet fuel into a bucket or pot. Add the liquid ingredients and allow the water to get absorbed.

2) Add the remaining dry ingredients and mix them in thoroughly with a spoon or spatula. Keep mixing until the pellets have broken down into sawdust.

3) Measure 9-10 oz (255-283 gms., or roughly 2.75 cups) of the resulting substrate into six clear food storage bags. Twist the bags loosely closed.

4) Heat about 3 inches of water to a rolling boil in a large pot or canning cauldron with some sort of rack or heat iffuser at the bottom. Place all of the bags at once into the boiling water, lowering them in

by holding the necks f the bags together.

5) Cover the pot and boil for 8 minutes.

6) Immediately remove all of the bags and float them on cold water in a wide pan, taking care not to let the ouths of the bags get into the water.

7) When the bags have cooled substantially, remove them from the water and tie off the mouths of the bags about n inch or so from the tops with twist ties.

8) When the bags have cooled completely, they are ready to inoculate.

Using the spawn

To use your bag spawn, break up the mycelium the day before you will inoculate, manipulating the bag to turn he clump of mycelium into lumps, and the lumps into smaller particles. Take care not to puncture the bag with our fingernails, or with the twist tie. The next day, when you are ready to pour out the spawn from these bags, e aware that the mouths of the bags are not sterile above the twist ties, so you shouldn't use them like pour pouts. Instead,

1) pull open the mouth of the bag by grasping from the outside surfaces

2) fold the mouth of the bag back on itself, and

3) push the spawn out while turning the bag inside out.

Preparation of Bulk Substrate

Bulk Substrate I:

Preparing straw with peroxide -- at room temperature

Next we come to a method for using peroxide to prepare straw for use as mushroom substrate. This method should be attractive to both the home grower and the commercial cultivator because the procedure can be carried out entirely at room temperature, with no heating and cooling step, and no caustic solution required. This makes substrate preparation very convenient and inexpensive, with no need for set-ups to heat large amounts of water or substrate, no problems with over-pasteurization, and no concerns about the speed of cooling. And in contrast to the hydrated lime soak method presented in Volume I, there is no problematic waste produced by the substrate preparation process, other than the natural "tea" that is normally produced by soaking straw in water. Finally, it should be possible to

prepare other similar "drainable" substrates such as bagasse, dried grasses, dried corn leaves, etc., in the same way. Materials of this kind are readily available in most parts of the world.
I have tested the protocol both with the elm oyster (H. ulmarius) and with the almond mushroom (Agaricus subrufescens), so similar species such as traditional oyster mushrooms (Pleurotus species), Portobellos, white button mushrooms, and Royal Sun Agaricus (Agaricus blazei) should grow well on straw prepared this way. More of a question mark is shiitake, only because it is typically grown on straw with a nitrogen supplement, and I haven't used a supplement to grow the elm oyster and the almond mushroom.

What about the enzymes?

Here I have to admit that my previous publications all argued that straw could not be usefully pasteurized by treatment with hydrogen peroxide solution. I reasoned that straw contains high levels of peroxide-decomposing enzymes in it (as do other similar substrates), and these enzymes would both destroy the peroxide in short order and protect the numerous mold spores in the straw from the peroxide. Now It turns out that straw nevertheless CAN be pasteurized with hydrogen peroxide. Yes, the peroxide is indeed destroyed by the enzymes in the straw in a relatively short time. But if we raise the peroxide concentration (compared to what I previously employed for pellet fuel preparation), and we tweak the chemistry of the peroxide solution slightly, the peroxide can still have a beneficial effect even in the brief time it survives contact with the straw. And although the peroxide itself does not linger to protect the straw from subsequent contamination, as it does in enzyme-free pellet fuel substrate, the peroxide nevertheless seems to transform the straw into a substrate that is favorable for the growth of mushroom mycelium, one that resists contamination even when the peroxide itself is gone. The peroxide apparently does this at least partly by way of a chemical reaction with the straw.

The protocol

Despite this complicated explanation, the protocol for preparing straw with peroxide is extremely simple. It goes like this:
1) Place your straw in a large soak vessel.
2) Fill the vessel with the appropriate cold solution (see below) to immerse the straw.
3) For chopped straw, soak about 4 hours at room temperature. For unchopped straw, soak for at least 28 hrs, or until the leachate takes on the color of a good tea.

4) Drain the straw thoroughly, until it is no longer drippy.
5)Remove the straw to your culture containers, mixing in spawn as you go.

Notes on straw preparation (keyed to the step numbers)**:**

1) If the soak vessel has a heavy or tight fitting lid, this can help keep the straw submerged in the solution.
2) The most effective solution I have found so far uses hydrogen peroxide at a concentration of at least 0.15%, combined with 10 mls. of vinegar per liter of soaking solution, or about 2.5 tablespoons vinegar per gallon (higher concentrations of vinegar did not work in mini-trials).
Curiously enough, an alternative which has proved almost equally effective in mini-trials is hydrated lime (calcium hydroxide, Masons' lime) in peroxide. But here you will use far less hydrated lime than called for by the hydrated lime soak detailed in Volume I. Instead of adding so much hydrated lime that you create something close to a saturated solution, which then creates disposal problems when you drain the straw, you will now add just enough hydrated lime to raise the pH a bit while the peroxide reacts with the straw. You'll use just 1/2-2/3 tsp. hydrated lime per gallon of 0.15% peroxide solution, or 0.4 - 0.5 gms. per liter of solution (higher
concentrations of hydrated lime did not work in mini-trials).
The solutions can be prepared with cold water from the tap, but the best bet is to use water that is not far below room temperature.
3) I recommend chopping the straw for best results. Chopping the straw promotes more efficient absorption of water, and the smaller particle size encourages faster mycelial growth upon inoculation. The wetting of the straw will proceed more quickly in warmer climates, more slowly in colder ones, so adjust your soak times accordingly.
4) How long you have to drain the straw depends on how much straw you are working with, but a couple of hours will probably be the minimum.
5) Straw can be mixed with spawn and loaded into plastic columns, for instance. Pasteurized gypsum, if added, can be mixed in at this stage as well. I don’t recommend adding nitrogen supplements, as most of the standard ones like bran will invite contaminants. But if you must have a supplement, I would suggest using drainable materials resembling straw, such as alfalfa, which can then be soaked in peroxide solution with the straw.

Bulk Substrate II:

An "add-and-stir" method for peroxide-compatible substrates

This next procedure allows you to prepare wood-based mushroom substrate at room temperature, virtually in a single step. Unlike the previous method for straw, this procedure does require that you use a starting material devoid of peroxide-decomposing enzymes. You can use wood pellet fuel, or any similar peroxide-compatible wood product such as composite logs, sawdust-based cat litter, kiln dried sawdust, or paper fiber pellets--just be sure the material is otherwise conducive to the growth of the mushroom species you want to cultivate. As with my earlier methods, the added peroxide will remain in the substrate and protect against airborne contaminants, so there should be no need for air filtration or sterile facilities. The resulting substrate should allow contaminant-free growth of any wood decomposing mushroom species, given an appropriate choice of additives, wood type, and growing conditions. In the first volume of **Growing Mushrooms** the **Easy** Way, I presented a procedure for preparing wood pellet fuel that required adding boiling water to the pellets as the first step. This served both to pasteurize the pellets and to break them down into sawdust rapidly. Peroxide solution, added only after the pellets had cooled, then served to kill heat resistant spores still present in the substrate and to protect the substrate from airborne
contaminants. This procedure works well for the home cultivator, but because of the two steps of liquid addition combined with the need for heating and cooling, it is rather awkward to scale up for commercial cultivation. The new procedure presented here avoids the awkwardness of the previous procedure by using a peroxide concentration about 10 fold higher than the previous method. At this level, the peroxide solution itself pasteurizes the pellet fuel at room temperature, so you need no heating and cooling, and you can add the peroxide with all of the water in one step of liquid addition. Moreover, you can add all your other additives like peroxide-compatible nitrogen supplements, lime and gypsum right along with the peroxide solution, so you have what is essentially an "add and stir" method of making ready-to-use mushroom substrate. It is not quite as **easy** as making pancakes from a mix, because you'll need to wait for the peroxide to pasteurize the substrate. But it is undoubtedly one of the simplest methods of preparing sawdust substrate yet devised.

The "add-and-stir" protocol

Here’s the new protocol applied to preparing pellet fuel at room temperature:
1) Clean and rinse a container (a boiling water rinse is not required).

2) Measure out your lime, peroxide-compatible nitrogen supplement, and gypsum if used, and have them at the ready (see Volume I for determining appropriate supplements and amounts).
3) Measure your pellet fuel into the container.
4) Measure into a separate container (very roughly) 6.5 quarts of water for every 10 lbs. pellet fuel (but see the note below). The water needs to be at least "room temperature" (68 degrees F) or slightly warm to the touch for proper breakdown of pellets made from dense woods like oak. As in previous protocols, the water also needs to be free of visible particulates.
5) To the water, add peroxide to reach a concentration of about 0.45%.
6) If you are using a pellet fuel that does not break down easily in water, such as that made from oak, add a teaspoon (5mls.) of baking soda to every 6.5 quarts of peroxide solution as well.
7) After stirring the baking soda into the peroxide solution, pour the solution into the pellet fuel; then add the other dry ingredients (I suggest doing it in this order so that the other dry ingredients don't get stuck on the bottom of the container).
8) Close the lid and let the pellets absorb most of the water (this may take 10-15 minutes).
9) Mix the substrate thoroughly by rolling the container.
10) Let the substrate sit in the closed container for at least 2 hours.
11) Mix the substrate thoroughly again. By this time, the substrate should be at least about half in the form of sawdust and half as the round remnants of pellets.
12) Inoculate the substrate and divide into bags.

Notes on the "add-and-stir" protocol:

As always, it is best to use woods favored by the mushroom species you want to grow. In general, avoid resinous woods like cedar and pine. You will probably have to experiment a bit to find the right amount of water to add, as this varies with the kind of wood your pellets are made of, and with the kind of substrate if you are not using wood pellets. You want to add enough water to break down the substrate to particles, but the material left at the bottom of the container (when you pour out the substrate into bags) ideally should be moist but not visibly wet. Pellets made from lighter woods will probably absorb more water per weight of pellets than those made from denser woods.
If you are using a substrate material that resists wetting, such as kiln dried sawdust, you can add a small amount (1/4 tsp. per 6.5 qts. water) of biodegradable dish detergent to speed up water absorption.
The high peroxide concentration used in this protocol is most easily accomplished with a peroxide

stock solution that is more concentrated than the usual drugstore product. I have used 1/3 cup of swimming pool peroxide (labeled 27%, but it actually tested at 34% by decomposition) for 6.5 quarts of water. You could also use food grade (35%) or similar solutions. Remember that these concentrated products are much more hazardous than the 3% solution. The liquid can cause burns, fires, or explosions, so it should be treated with considerable respect. Read the warning label and act accordingly. The substrate can be mixed the first time with non-pasteurized implements, if this is more convenient than rolling the container. After this first mixing, however, non-pasteurized implements should be kept out of the substrate, and only pasteurized (i.e. boiling-water rinsed) implements should be used.

What about making spawn by the "add-and-stir" approach? The verdict isn't in yet on whether this will give spawn that is clean enough for reliable use. But you are welcome to try it. Just mix up some medium as for Eight Minute spawn above, or as spelled out in Volume I (Ten Minute spawn), but increase the peroxide concentration to 0.45%. If you are using jars, wet the cardboard disks inside the lids with3% peroxide. Then let the spawn sit, closed up in its container, for at least two hours (overnight might be safer) before inoculating.

Bulk Substrate III: Preparing bulk substrate by baking

One way to prepare "raw" substrates so that you can use them with the peroxide method like you would use pellet fuel is to bake the substrates in an oven. Baking eventually will to destroy the peroxide-decomposing enzymes in any substrate. For instance, although raw sawdust is rich in peroxide-decomposing enzymes, kiln-dried sawdust (that is, sawdust from milling of kiln-dried lumber) has almost no peroxide-decomposing activity left in it. Although baking will be a less-than-ideal procedure for many mushroom growers because of the energy costs, lack of oven space, and the odors generated by the procedure, it nevertheless may come in quite handy for some. If you are a hobbyist who only has an ordinary kitchen pressure cooker, baking in your oven may allow you to prepare a larger batch of substrate than you could otherwise manage. And in areas where sunlight is plentiful, you could bake your substrate outdoors in a large solar oven built especially for this purpose, solving the problems of energy cost, odors, and oven space all at once.

The baking process

You will have to work out for yourself the exact details of temperature and time for baking the

particular

substrate you have in mind, given the variety of possible substrates with different moisture contents and particle sizes. In general, however, you will bake at 275-300 degrees F (about 150 degrees C) for several hours, or long enough to raise the temperature inside the substrate to 250 degrees F (121 degrees C) for at least 20 minutes.

Then let the substrate continue baking after turning off the oven. Wet materials will take longer to reach the necessary temperature. Wide pans will facilitate heat penetration.

To test your substrate to see whether your baking has had the desired effect,

1) remove a small amount and place it in a cup or small jar

2) add enough 3% peroxide solution to cover the substrate

3) add a drop of detergent to encourage the solution to penetrate.

If you see no more than a thin, fine fizz on the surface after fifteen minutes, you are probably in the clear.

Using baked substrate

Once your substrate has been sufficiently baked, you can cool it down and store it for later use; or you can use it immediately. Either way, you will have to experiment to determine what amount of water your substrate will absorb successfully, and what amount of moistened substrate will fit comfortably into your container.

I will assume your substrate is a non-drainable, porous material like sawdust (most drainable materials can be prepared without baking by following the protocol for straw above). Then you can prepare it for use following the "add-and-stir" protocol above. You just will not need to concern yourself with the parts of that protocol that have to do with getting the wood pellets to break down into sawdust. Instead, all you have to do is:

1) mix your substrate with peroxide solution and whatever other additives you use., and

2) let the mixture sit for a couple of hours to give the peroxide time to pasteurize it.

If your substrate is a drainable material, like wood chips, the protocol is even simpler. You will just need to soak the material in peroxide solution until it has reached a workable moisture content. Then it will be ready to inoculate.

If the cost of peroxide is a significant concern in your locale, you can reduce the final peroxide concentration by a factor of eight by heat-pasteurizing both your substrate mixture and the water you use to add your peroxide. To go this route, you will need to

1) clean your container and rinse it with boiling water

2) divide your total water in half
3) add half of it as boiling water to the substrate mixed with your lime, peroxide-compatible nitrogen supplement, and gypsum (if used)
4) boil and cool the other half of your water in a pot with a lid
add enough peroxide to the boiled, cooled water to give a concentration of about 0.1% (a little over 1/2 cup—136 mls.--of 3% peroxide per gallon , or 36 mls. per liter)
5) when the substrate has cooled somewhat add the peroxide solution to the substrate and mix thoroughly
6) let the mixture cool completely, then inoculate.
(A possible alternative to boiling and cooling water for the peroxide solution is to use water that has been purified by reverse osmosis, since such water is sterile as it emerges from the membrane).

Conclusion

The procedures I've spelled out in this supplement give a glimpse of the tremendous variety possible in mushroom growing techniques. At any given time, I usually have at least a few ideas for new tricks that I haven't had a chance to test, and I know from the correspondence I get that many of my readers have their own
fascinating ideas as well. I hope this volume stimulates further creativity in this rapidly growing field.

About the Author

Rush Wayne holds a Masters degree in Biochemistry and Molecular Biology from Harvard University and a Ph. D. in Biochemistry from the University of California at Berkeley. He was first exposed to the elements of mushroom growing during his graduate work in the 1970’s but did not begin **growing mushrooms** in earnest until he began to implement the innovations contained in this manual in 1993. Instructions for his peroxide method of **growing mushrooms** are now in the hands of mushroom growers in over 65 countries around the
world.